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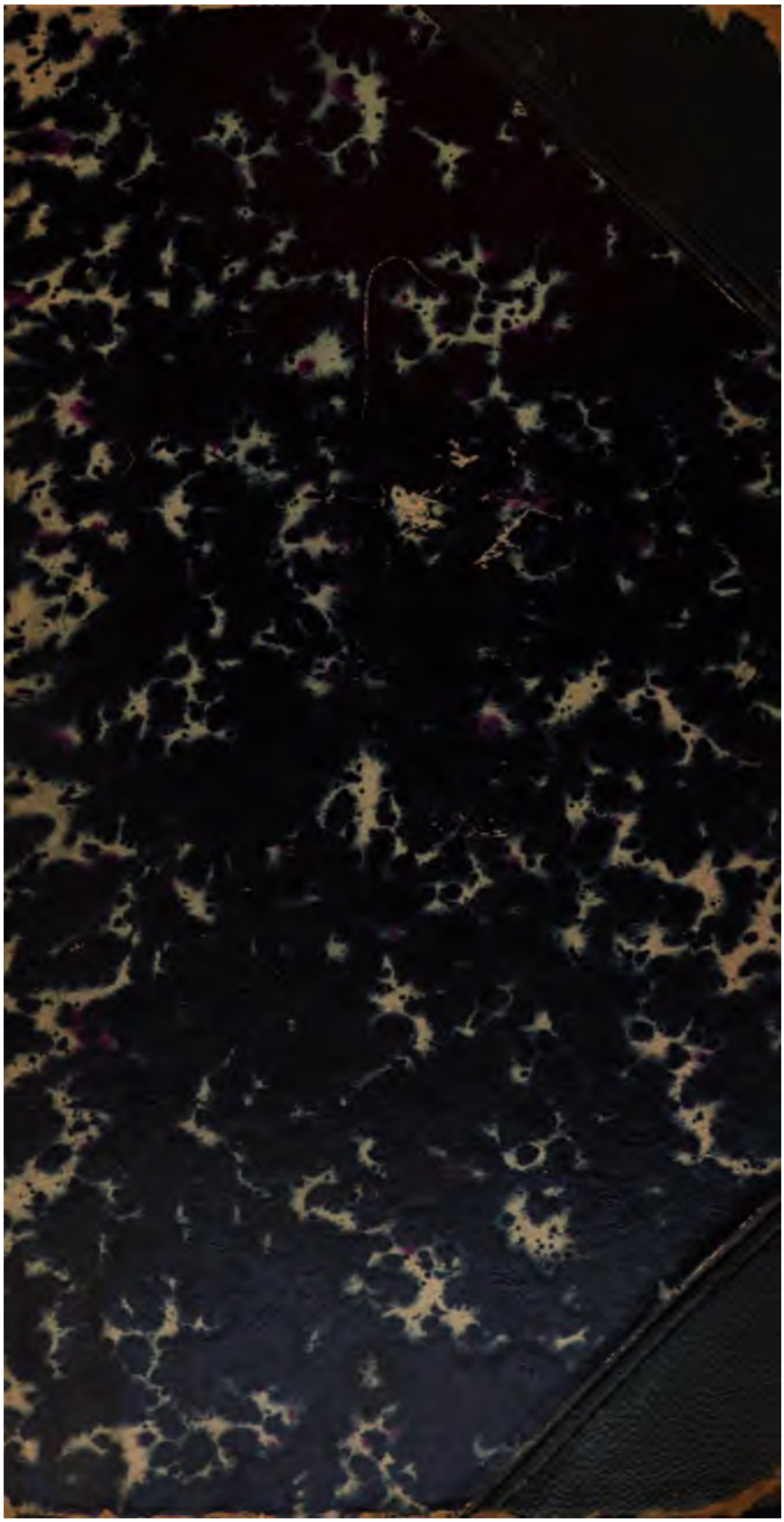

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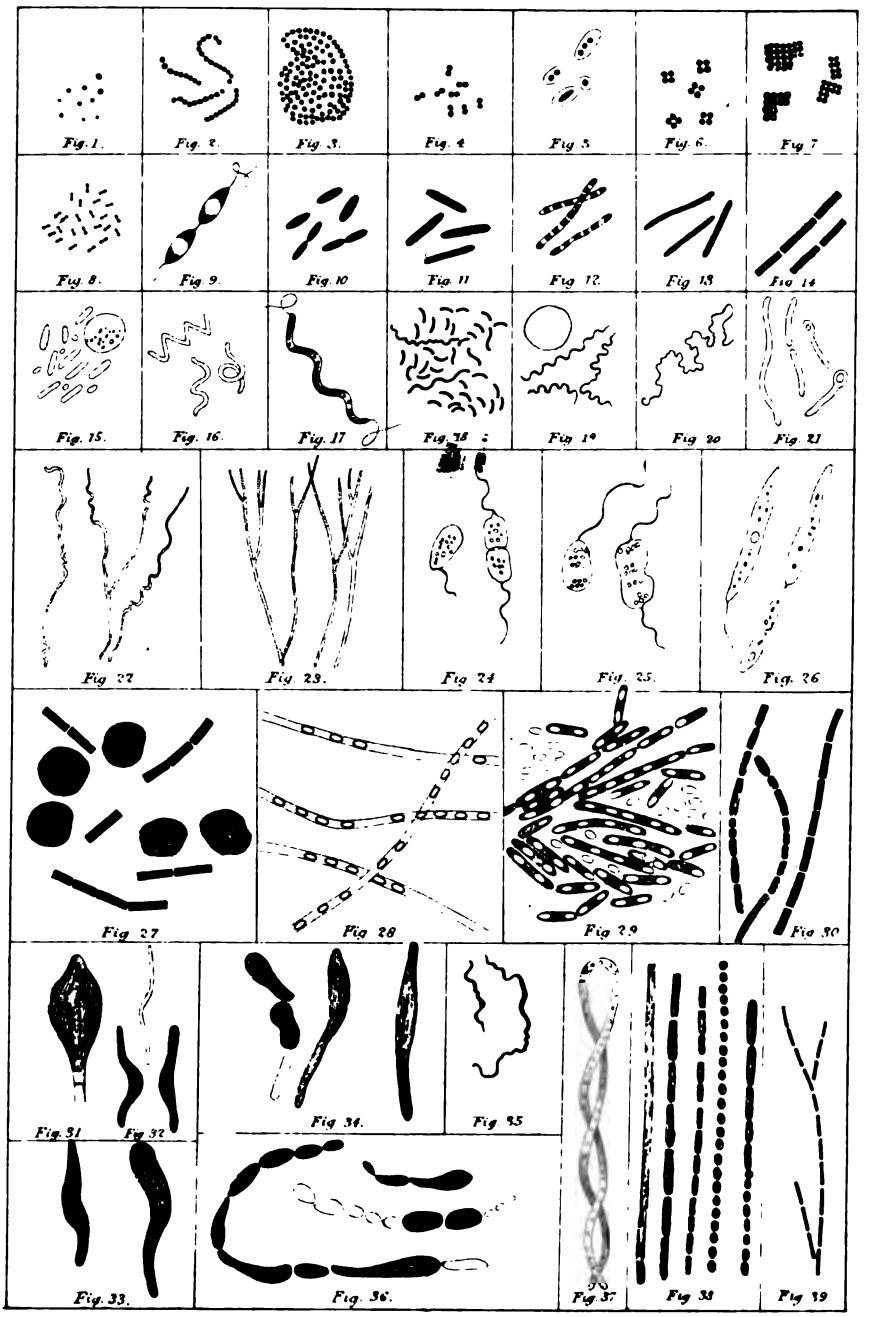

BACTERIA. SCHIZOMYCETES OR FISSION-FUNGI.

MANUAL

OF

BACTERIOLOGY

BY

EDGAR M. CROOKSHANK

M.B. (LOND.), F.R.M.S.

DEMONSTRATOR OF PHYSIOLOGY, KING'S COLLEGE, LONDON

SECOND EDITION, REVISED AND CONSIDERABLY ENLARGED

ILLUSTRATED WITH COLOURED PLATES AND WOOD ENGRAVINGS

LONDON

H. K. LEWIS, 136 GOWER STREET, W.C.

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To

SIR JOSEPH LISTER, BART., M.B., F.R.S.,

WHO HAS CREATED A NEW EPOCH IN

MEDICINE AND SURGERY,

BY APPLYING A KNOWLEDGE OF MICRO-ORGANISMS

TO THE TREATMENT OF DISEASE,

This Work is Dedicated

AS A

TOKEN OF ADMIRATION AND RESPECT

BY THE AUTHOR.

PREFACE

TO THE SECOND EDITION.

THE fact that a new edition of this manual was called for a few months after its publication, has induced the author to extend its scope in the hope of adding to its usefulness.

The work has not only been revised throughout and brought up to date, but, in order to admit of a more concise arrangement of the species, the Systematic part has been recast.

Additional chapters have been written upon the General Morphology and Physiology of Bacteria, upon Antiseptics and Disinfectants, and Immunity.

Seventy-three illustrations have been added. Those not duly acknowledged as coming from other sources were drawn on the wood by the author from his own preparations.

A list of references to works on Bacteriology, which was not ready for the first edition, has now been completed and extended. It has no pretension to be a complete bibliography, but being arranged as much as possible in accordance

with the chapters, and in chronological order, may be useful to those seeking further details. No doubt Professor Baumgarten's *Jahresbericht*, the first number of which has been issued this year, will be found a valuable guide to current literature in the future.

The author desires again to express his acknowledgments to Professor Gerald Yeo and Mr. Herroun, of King's College, London.

EDGAR M. CROOKSHANK.

24, MANCHESTER SQUARE, W.,
December, 1886.

PREFACE

TO THE FIRST EDITION.

A BRANCH of study, which has opened fresh paths in pathology and therapeutics, is so important that it may in time become a more essential part of the medical curriculum, and is perhaps worthy of an elementary handbook, which shall include a systematic sketch of the genera and species of micro-organisms, as well as the methods employed in the investigation of their life-histories.

Having myself experienced the want of a practical aid to the study of Bacteriology by the methods introduced by Professor Koch, I thought that it might be useful to those wishing to commence this study by these recent methods, if I embodied my notes made in different laboratories in the form of a Manual for Students. The work is thus intended to help the student beginning the study of a subject, the literature of which, in English, is for the most part diffused in numerous periodicals.

The methods of "pure cultivation" of Bacteria will, after all, be found to be remarkable for their extreme simplicity, and can be easily mastered by

the careful clinical worker, to whom it is hoped this little book may also prove useful.

I take this opportunity of expressing my best thanks to Professor Virchow, who materially furthered my work in the Pathological Institute of Berlin by kind advice and generous assistance.

I am most grateful to Dr. Babes, of Budapest, for his ever-ready co-operation; and to Professor Johne, of Dresden, who also placed his laboratory at my disposal, and to whom I am particularly indebted for much of the material from which the microscopical preparations were made.

I would also wish gratefully to acknowledge the great interest and courteous assistance shown me on the part of Dr. Hauser, of Erlangen; Professor Pettenkofer, Professor Bollinger, and Dr. Büchner, of Munich; and the officials of the new Hygienic Laboratory in Berlin.

The original drawings from which the coloured plates of test-tube- and potato cultivations are reproduced were made by my wife from cultivations prepared especially for the purpose, and selected as typical. The coloured plates of the microscopic appearances are reproductions of my own drawings, from specimens I had recently prepared. The drawings were made from parts selected as most characteristic from a great number

of preparations, which have been since demonstrated at the meeting of the Royal Microscopical Society, on November 25th.

All the plates have been most faithfully and skilfully reproduced by Messrs. Vincent Brooks, Day, & Son.

In conclusion, I owe much to Professor Gerald F. Yeo, of King's College, London, for many valuable criticisms; and to my colleague, Mr. Herroun, for his kindness in reading the proof-sheets.

EDGAR M. CROOKSHANK.

24, MANCHESTER SQUARE, W.,
December, 1885.

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22. *Cladothrix Førsteri* (after Cohn).
23. *Cladothrix dichotoma* (after Cohn).
24. *Monas Okenii* (after Cohn).
25. *Monas Warmingii* (after Cohn).
26. *Rhabdomonas rosea* (after Cohn).
27. From a cover-glass-preparation of blood from the spleen of a mouse which had died of anthrax; stained with fuchsine (Zeiss' $\frac{1}{18}$ o.i. Oc. 4).
28. From a drop-cultivation of *Bacillus anthracis* (Zeiss' $\frac{1}{18}$ o.i. Oc. 4).
29. From a cover-glass impression-preparation of a potato-cultivation of *Bacillus anthracis* (Zeiss' $\frac{1}{18}$ o.i. Oc. 4).
30. From a preparation of *Bacillus anthracis*, cultivated in nutrient gelatine (torula-form).
31. Involution-form of *Crenothrix* (after Zopf).
32. Involution-forms of *Vibrio serpens* (after Warming).
33. Involution-forms of *Vibrio rugula* (after Warming).
34. Involution-forms of *Clostridium Polymyxa* (after Prazmowski).
35. Involution-forms of the *Spirillum choleræ Asiaticæ*, from an artificial cultivation.
36. Involution-forms of *Bacterium aceti* (after Zopf and Hansen).
37. Spirulina-form of *Beggiatoa alba* (after Zopf).
38. Various thread-forms of *Bacterium merismopedioides* (after Zopf).
39. False-branching of *Cladothrix* (after Zopf).

BACTERIOLOGY.

CHAPTER I.

INTRODUCTORY.

The researches of Pasteur into the *rôle* played by micro-organisms in the processes of fermentation and putrefaction, and in diseases such as anthrax, the silkworm malady, pyæmia, septicæmia, and chicken cholera, have invested the science of Bacteriology with universal interest and vast importance. The further researches of the practical mind of Lister, with the resulting evolution of antiseptic surgery, have demonstrated the necessity for a more intimate acquaintance with the life-history of these micro-organisms; while the more recent investigations which have established the intimate connection between bacteria and infectious diseases, and more especially the discovery by Koch of the tubercle and cholera bacilli, have claimed the attention of the whole thinking world.

The scientific importance of these latter dis-

coveries is evidenced by the fact that in Germany medical men were summoned from all parts of the country to Berlin to attend a course of instruction in the latest methods employed in the investigation of bacteria. In this way the methods of *pure cultivation* devised by Koch, and the means of recognising the cholera bacillus, are being widely disseminated.

To a medical man, those bacteria which are connected with disease, and more especially those which have been proved to be the *causa*, if not the actual *materies morbi*, are of predominant interest and importance. It is, however, impossible by localising one's knowledge to pathogenic species to thoroughly understand the life-history of these particular forms, or to be able to grasp and appreciate the various arguments and questions that arise in comparing their life-history with the progress of disease.

It is not sufficient to know only how to recognise and artificially cultivate a bacterium associated with disease; we must endeavour to establish the exact relationship of the bacterium to the disease in question. To ascertain beyond all doubt whether a micro-organism is actually the *causa causans* of a disease, Koch has laid down the following postulates:—

a. The micro-organism must be found in the blood, lymph, or diseased tissues of man, or animal, suffering from, or dead of, the disease.

b. The micro-organisms must be isolated from the blood, lymph, or tissues, and cultivated in suitable media,—*i.e.*, outside the animal body. These *pure cultivations* must be carried on through successive generations of the organism.

c. A pure-cultivation thus obtained must, when introduced into the body of a healthy animal, produce the disease in question.

d. Lastly, in the inoculated animal the same micro-organism must again be found.

These points would naturally suggest a sequence in the various processes which must be adopted in a practical study of micro-organisms associated with disease. Inasmuch, however, as these processes embrace those which are employed in the isolation, cultivation, etc., of non-pathogenic species, we shall, in studying the bacteria as a whole, adopt the order suggested. After an introduction to the apparatus commonly employed in a bacteriological laboratory, we shall describe the methods of examining liquids, tissues, etc., and the means of recognising micro-organisms. Then will follow the methods of isolating these micro-organisms from such liquids, tissues, etc., and of carrying on pure cultivations in nutrient media. Lastly, we shall refer briefly to experimental researches on the living animal, and the means of isolating micro-organisms from the liquids and tissues of the body after death.

In Part II. will be found chapters upon the General Biology of bacteria, and in Part III. a

chapter upon their classification, followed by a description of each species, more particularly of those of pathological interest, with a detailed account of the special methods of examination and of staining employed for demonstrating the different species.

In the Appendix a descriptive list of important yeasts and moulds will be given, with any special technique required in their case. Yeasts and moulds are constantly encountered in the special methods for examining bacteria in air, soil, and water, and several are of interest in being, like many bacteria, micro-organisms associated with disease. A short account is also given of the Flagellated Protozoa, which have been found to occur in the blood of animals. To the pathologist these are of interest owing, more especially, to the discovery of closely allied micro-parasites in the blood in cases of malaria.

The special methods just referred to, with description of the apparatus employed, and a Chronological Bibliography, are also comprised in the Appendix.

CHAPTER II.

APPARATUS, MATERIAL, AND REAGENTS EMPLOYED IN A BACTERIOLOGICAL LABORATORY

(A) HISTOLOGICAL APPARATUS.

Microscope.—For the investigation of micro-organisms a good microscope with oil-immersion system and a condenser, such as Abbé's, is essential. Such instruments are supplied by Leitz, Zeiss, and Hartnack in Germany, and Powell and Lealand in England. Zeiss' microscope, with $\frac{1}{12}$ and $\frac{1}{18}$ oil-immersion lenses, or Powell and Lealand's with $\frac{1}{12}$ and $\frac{1}{25}$, is recommended for investigators; while Leitz', with $\frac{1}{12}$, is a serviceable and economical one for students.* In addition to the usual microscopic fittings, Zeiss supplies a micrometer eyepiece, with directions for use. Some such arrangement is essential for the measurement of bacteria. Other accessories to the microscope are—

A large bell-glass for covering the microscope when not in use.

About a foot square of blackened plate-glass.

* Leitz' with $\frac{1}{12}$ costs about £15; Zeiss', with the same, £30, and with $\frac{1}{18}$, £20 more. Refer to foot-note on p. 44.

A white porcelain slab of the same size.

Glass bottles with ground glass stoppers, for alcoholic solutions of aniline dyes, etc.

Glass bottles with funnels, for aqueous solutions of the dyes, and others provided with pipettes.

A small rod-stoppered bottle of cedar oil. This is recommended by Zeiss in preference to other oils for his immersion lenses.

Set of small glass dishes or capsules, and watch glasses, for section staining, etc.

Stock of best glass slides, in packets of fifty.

Several boxes of round and square thin cover-glasses, in various sizes, of the best quality.

Needle-holders, with a couple of platinum needles, and a packet of ordinary sewing needles.

Glass rods drawn out to a fine point; useful for manipulating sections when acids are employed.

Copper lifters, preferably plated.

One pair of small brass or spring-steel platinum-pointed forceps, for holding cover-glasses.

One pair of brass tongs.

Collapsible tubes for containing Canada-balsam; very serviceable for transport and general use.

Turn-table, used in preparing slides with rings, for mounting preparations of Aspergillus, etc.

Boxes for preparations, book-form.

Tickets and labels, various sizes.

Soft rags or old pocket handkerchiefs, for removing cedar oil after use of immersion lens, cleaning cover-glasses, etc.

Chamois leather for wiping lenses.

Microtome.—Schanze's is much in favour in Germany, but Jung's, of Heidelberg,* though a

* Price lists may be obtained from any of the above-mentioned firms, from which an idea of the instruments can be formed, and a comparison of the prices made.

somewhat cumbrous instrument, is much to be preferred. Smaller accessories, which should be within reach, are—

A small can of sewing machine oil.

A soft rag and chamois leather, for wiping the knives immediately after use.

Stone and leather for setting and sharpening the same.

Two or three camel's hair brushes.

A Freezing Microtome, such as Williams' or Roy's, and a Valentin's Knife, are useful for the examination of tissues in the fresh state, but otherwise are supplanted by the above.

(B) REAGENTS AND MATERIAL EMPLOYED IN THE PROCESSES OF HARDENING, DECALCIFYING, EMBEDDING, FIXING, AND CUTTING OF TISSUES.

Alcohol, absolute.

Bergamot oil.

Celloidin.

Dissolved in equal parts of ether and alcohol.

Cork, or stock of ready-cut corks.

Ebner's solution. A mixture in the following proportions:—

Hydrochloric acid . . .	5·
Alcohol	100
Distilled water	20
Chloride of sodium . . .	5·

Gelatine.

Melted in a small porcelain capsule and set aside ready to be re-melted when required for use.

Glycerine-gelatine (Klebs).

Best well-washed gelatine . .	10
Add distilled water, allow gelatine to swell up, pour off excess of water, melt gelatine with gentle heat, add	
Glycerine	10
Lastly a few drops of phenol for preservation	

Gum.

Kleinenberg's solution.

Saturated watery solution of picric acid	100
Strong sulphuric acid . . .	2
Filter and add	
Distilled water	300

Muller's fluid.

Bichromate of potash . . .	2
Sulphate of sodium	1
Distilled water	100

Osmic acid.

Distilled water	100
Osmic acid	·5

Paper trays.

Paraffin.

Spermaceti.

Xylol.

Hardening and decalcifying solutions should be kept in stock in quantities according to requirement. A jacket of brown paper should be pasted round a well stoppered bottle to contain osmic acid to efficiently protect it from light, and it should be kept in a cool place. The celloidin solution may be kept in stock in a wide-mouthed glass bottle, from which small wide-mouthed bottles may be filled according to the number required. To put several pieces of different tissues in the same bottle leads to confusion.

(C) REAGENTS FOR EXAMINING AND STAINING MICROSCOPICAL PREPARATIONS.

1. **Acetic acid, strong.**
2. **Alcohol—absolute.**
3. **Alcohol—60 per cent.**
4. **Alcohol—acidulated.**

Alcohol	100
Hydrochloric acid	1

5. **Alum carmine** (Grenacher).

Carmine	1
Five per cent. solution of alum .	100

Boil twenty minutes, filter when cold.

6. **Ammonia, strong.**

7. **Aniline.**

8. **Aniline water.**

Distilled water	100
Aniline	5

Shake well and filter emulsion.

9. **Bismarck brown.**

(*a*) Concentrated solution in equal parts of glycerine and water.

(*b*) Aqueous solution.

Bismarck brown	2
Alcohol	15
Distilled water	85

10. **Borax carmine** (Grenacher).

Borax	2
Carmine	·5
Distilled water	100

To the dark purple solution add a 5 p. c. sol. of acetic acid until a red colour is produced; set aside 24 hours, filter, and add a drop of carbolic acid.

11. Cedar oil.

12. Eosin.

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Distilled water	100
Eosin	5

13. Ether.

14. Fuchsine.

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Fuchsine	2
Alcohol	15
Water	85

15. Gentian violet.

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Gentian violet	2·25
Distilled water	100

16. Gibbes' solution, for double staining.

Take of

Rosaniline hydrochlorate	2
Methylene blue	1

Triturate in a glass mortar,

Dissolve aniline oil	3
In rectified spirit	15

and add slowly to the above.

Lastly, slowly add distilled water .	15

Keep in stoppered bottle.

17. Glycerine, pure.

18. Hæmatoxylin solution.

Hæmatoxylin	2
Alcohol	100
Distilled water	100
Glycerine	100
Alum	2

19. Iodine solution.

Iodine, pure	1
Iodide of potassium . . .	2
Distilled water	50

20. Iodine solution (Gram).

Iodine	1
Iodide of potassium . . .	2
Distilled water	300

21. Lithium-carmine solution (Orth).

Saturated solution of carbonate of lithium	100
Carmine	2·5

22. **Magenta solution** (Gibbes).

Magenta	2
Aniline oil	3
Alcohol (Sp. Gr. ·830) . . .	20
Distilled water.	20

23. **Methylene blue.**

(*a*) Concentrated alcoholic solution.

(*b*) Aqueous solution.

Methylene blue	2
Alcohol	15
Water	85

(*c*) Koch's solution.

Concentrated alcoholic solution of methylene blue	1
Ten per cent. potash solution . .	2
Distilled water	200

(*d*) Löffler's solution.

Concentrated alcoholic solution of methylene blue	30
Solution of potash 1—10,000 . .	100

24. **Methyl violet.**

(*a*) Concentrated alcoholic solution.

(*b*) Aqueous solution.

Methyl violet	2.25
Distilled water	100

(*c*) Koch's solution.

Aniline water	100
Alcoholic solution of methyl violet	11
Absolute alcohol	10

25. **Neelsen's solution.**

Dissolve fuchsine . . .	1
In a 5 per cent. watery solution of carbolic acid	100
Add alcohol	10

26. **Nitric acid, pure.**

27. **Orseille** (Wedl).

Dissolve pure ammonia-free orseille in

Absolute alcohol . . .	20
Acetic acid	5
Distilled water	40

until a dark red liquid results: filter.

28. **Picric acid.**

(*a*) Concentrated alcoholic solution.

(*b*) Saturated aqueous solution.

29. **Picro-carmine** (Ranvier).

Carmine	1
Distilled water	10
Solution of ammonia	3
Triturate, add cold saturated solution of picric acid	200

30. **Picro-lithium-carmine** (Orth).

To above mentioned Lithium-carmine solution add	
Saturated solution of picric acid	2·3

31. **Potash solution.**

(*a*) 1 to 3 per cent.

(*b*) 10 ,, ,,

(*c*) 33 ,, ,,

32. **Safranine.**

(*a*) Concentrated alcoholic solution.

(*b*) Watery solution . 1 per cent.

33. **Sulphuric acid, pure.**

34. **Salt solution** . 0·8 per cent.

35. **Turpentine.**

36. **Vesuvin.**

(*a*) Concentrated alcoholic solution.

(*b*) Watery solution.

Water, distilled.

Water, sterilised.

Distilled water can be kept for use in a wash bottle, or far better in a siphon apparatus. Sterilised water is convenient in plugged sterile test-tubes which may be kept close at hand in a beaker, or tumbler, with a pad of cotton wool at the bottom. The *numbered* reagents can be conveniently arranged on shelves within easy reach. Alcoholic solutions of the aniline dyes and other special preparations should be kept in bottles with ground glass stoppers. Aqueous solutions of the dyes may be kept in bottles with funnel filters, and the solution filtered before use. To both aqueous and alcoholic solutions a few drops of phenol, or a crystal of thymol, should be added as a preservative. For the rapid staining of cover-glass preparations, it is convenient also to have the most frequently used stains (fuchsine, methyl-violet) in bottles provided with pipette-stoppers.

(D) REAGENTS FOR MOUNTING AND PRESERVING PREPARATIONS.

Acetate of potash.

Concentrated solution.

Asphalte lac.

Canada balsam.

Dissolved in xylol.

Glycerine gum (Farrant's solution).

Glycerine.
Water.
Saturated solution of arsenious acid.
Equal parts, mix and add of picked gum arabic half a part.

Hollis' glue.

Zinc-white.

(E) DRAWING AND PHOTOGRAPHIC APPARATUS.

Camera Lucida.—The camera lucida of Zeiss is an excellent instrument, though many prefer the pattern made by Nachet of Paris. Combined with the use of a micro-millimeter objective it affords also a simple method for the measurement of bacteria.

For drawing macroscopical appearances, and for illustrating microscopical specimens with or without the use of a camera lucida, the following materials should be within reach :—

Pencils.
Etching Pens.
Prepared Indian Ink.
Water-colour Paints and Brushes.
Ordinary and tinted drawing paper and other usual accessories.

Photo-micrographic Apparatus. — Zeiss of Jena, Seibert & Kraft of Wetzlar, Nachet of Paris,

and Swift & Son of London, may all be recommended for constructing an arrangement in which the photographic camera is combined with the microscope.

For illumination either sunlight or artificial light may be employed. In the case of sunlight a heliostat is necessary to procure the best results, but as sunlight is not always available by day, and it is also more convenient for many to work at night, it is better to have recourse altogether to artificial light. Excellent results may be obtained with an ordinary paraffine lamp, or with magnesium, oxycalcium, or electric light. Specimens are preferably stained yellow, brown, or black, and for mounting the preparations Koch* recommends a saturated solution of acetate of potash; but there is little or no objection to the use of Canada balsam dissolved in xylol. Hauser,† who employed the electric light, obtained some excellent pictures of preparations mounted in balsam. Van Ermengem‡ first recommended the isochromatic dry plates, and produced most successful results with the lime-light from objects stained with fuchsine and methyl-violet. The author also has investigated the applicability of photographic processes for illustrating micro-

* Koch, *Verfahren zur Untersuchung zum Conserviren und Photographiren der Bacterien.* 1877.

† Hauser, *Über Fäulniss Bacterien und deren Beziehungen zur Septicämie.* 1885.

‡ Van Ermengem, *Bull. de la Soc. Belge de Microscopie*, No. X., pp. 170-2. 1884.

organisms. Numerous preparations have been satisfactorily depicted by means of the isochromatic plates without any reference to the staining reagents employed. For a full description of the apparatus and methods employed the reader is referred to the author's publication.*

(F) STERILISATION APPARATUS.

Steam-Steriliser.—A cylindrical vessel of tin about half a metre or more in height, jacketed with thick felt, and provided with a conical cap or lid (Fig. 1). The lid is also covered with felt, has handles on either side, and is perforated at the apex to receive a thermometer. Inside the vessel is an iron grating or diaphragm about two-thirds the way down, which divides the interior into two chambers—the upper or "steam chamber," and the lower or "water-chamber." A gauge outside marks the level of the water in the lower chamber; this should be kept about two-thirds full. The apparatus stands upon three legs, and is heated from below with two or three Bunsen, or better, a Fletcher's

FIG. 1.—KOCH'S STEAM-STERILISER.

* *Photography of Bacteria.* 1887.

burner. It is employed for sterilising nutrient media in tubes or flasks, for cooking potatoes, or hastening the filtration of agar-agar. When the thermometer indicates 100° C. the lid is removed, and test-tubes are lowered in a wire basket by means of a hook and string, and the lid quickly replaced. Potatoes or small flasks are lowered into the cylinder in a tin receiver with a perforated bottom, which rests upon the grating and admits of its contents being exposed to the steam.

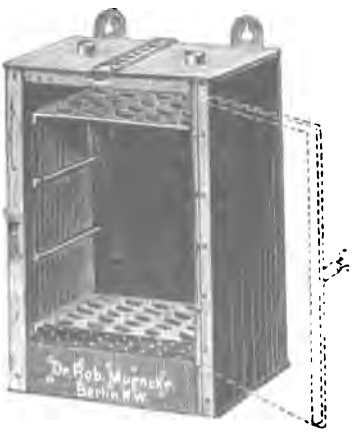


FIG. 2.—HOT-AIR STERILISER.

Hot-air Steriliser.—A cubical chest of sheet iron with double walls, supported on four legs; it may also be suspended on the wall of the laboratory, with a sheet of asbestos intervening (Figs. 2 and 3). It is heated with a rose gas-burner from below, and the temperature of the interior indicated by a thermometer inserted through a hole in the roof; in a second

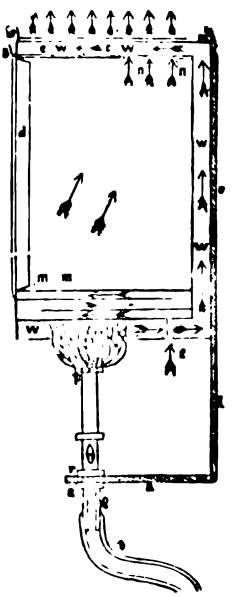


FIG. 3.—SECTION OF HOT-AIR STERILISER.

opening a gas regulator can be fixed. Test-tubes, flasks, funnels, cotton wool, etc., may be sterilised by exposure to a temperature of 150° C. for an hour or more.

(G) APPARATUS AND MATERIAL FOR PREPARING AND STORING GELATINE-, AND AGAR-AGAR-PEPTONE-BROTH.

Water-bath.—A water-bath on tripod stand is required for boiling the ingredients of nutrient jellies and for general purposes. The lid may be conveniently composed of a series of concentric rings, so that the mouth of the vessel may be graduated to any size required.

Test-tube Water-bath. — This consists of a circular rack for test-tubes within a water-bath. It is sometimes employed instead of the steam cylinder for sterilising nutrient jelly in tubes by boiling for an hour, for three successive days.

Hot-water Filter. — A copper funnel with double walls, the interspace between which is filled with hot water. A glass funnel fits inside the copper cone, the stem of the glass funnel passing through and being tightly gripped by a perforated caoutchouc plug, which fits in the opening at the apex of the cone. The water in the cone is heated by applying the flame of a

burner to a tubular prolongation of the water chamber. In a more recent model, as represented in Fig. 4, this prolongation is dispensed with, and the temperature is maintained by means of a circular burner which acts at the same time as a funnel ring.

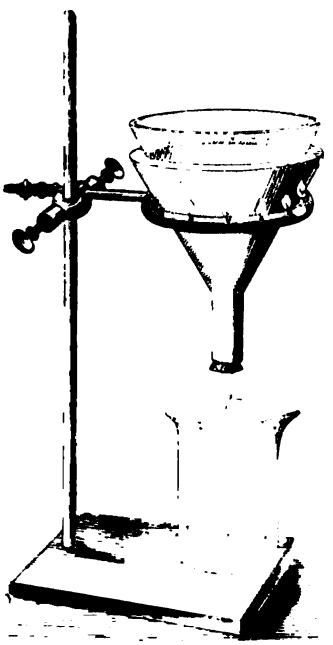


FIG. 4.
HOT-WATER FILTERING APPARATUS WITH RING BURNER.

Glass Vessels. — A number of glass vessels should be kept in stock according to requirements.

Bohemian hard glass flasks are employed in several sizes, for boiling nutrient media. The conical forms are especially used in the larger sizes for storing nutrient jelly.

Glass funnels large and small are necessary, not only in the processes of preparing nutrient jelly, but for filtering solutions of aniline dyes and for general purposes.

A liberal supply of test-tubes should always be kept in stock, as they are not only employed for the tube-cultivations, but can be conveniently used for storing bouillon, sterilised water, etc.

Cylindrical glasses graduated in cubic centimetres, 10 ccm., 100 ccm., 500 ccm., are required for measuring the liquid ingredients of nutrient jelly, and also in preparing the various staining solutions.

A large wide-mouthed glass jar, with a glass cover, is extremely useful. It must be padded at the bottom with cotton wool for containing a stock of tubes of sterilised nutrient jelly, and should be placed within reach on the working table.

Balance and Weights. — A balance, with large pans and set of gramme weights, is constantly required.

Cotton Wool.—The best or "medicated" cotton wool should be procured.

Gelatine. — The gelatine for bacteriological purposes must be of the very best quality (gold label).

Agar-Agar.—This is also called Japanese Isinglass; it consists of the shrivelled filaments of certain Algæ (Gracilaria lichenoides and Gigartina speciosa).*

Peptonum Siccum (Savory and Moore).

Table Salt.—Prepared table salt can be obtained in tins or packets.

Litmus Papers.—Blue or red litmus paper in cheque books, for testing the gelatine mixture, etc.

* Hueppe, *Die Methoden der Bakterien Forschung.* 1885.

Carbonate of Soda.—A bottle, containing a saturated solution of carbonate of soda, and provided with a pipette-stopper, may be kept, especially for use in the preparation of nutrient jelly.

Lactic Acid.

Filter Paper.—For filtering gelatine stout Swedish filter paper of the best quality is recommended.

Flannel or Frieze.—This is employed as a substitute for, or combined with, filter paper in the preparation of nutrient agar-agar.

(H) APPARATUS FOR EMPLOYMENT OF NUTRIENT JELLY IN TEST-TUBE AND PLATE CULTIVATIONS.

Wire Cages.—These cages or crates are used for containing test-tubes, especially when they are to be sterilised in the hot-air steriliser; or for lowering tubes of nutrient jelly into the steam steriliser, etc. (Fig. 5).

FIG. 5.—WIRE CAGE FOR TEST-TUBES.

Test-tube Stands.—The ordinary wooden pattern, or the metallic folding stands, are called into use for holding cultivations. Pegged racks are also recommended for draining test-tubes after washing.

Caoutchouc Caps.—These are caps for fitting over the cotton wool plugs, and may be used in different sizes for test-tubes and stock-flasks.

Platinum Needles.—A platinum needle for inoculating nutrient media, examining cultivations, etc., consists of two or three inches of platinum wire, fixed to the end of a glass rod. Several of these needles should be made, with platinum wire of various thicknesses. A piece of glass rod, about seven inches long, is heated at the extreme point in the flame of a Bunsen burner, and a piece

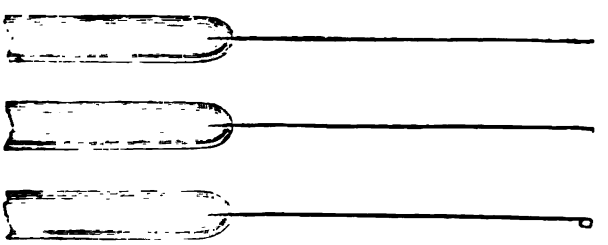


FIG. 6.—PLATINUM NEEDLES.

of platinum wire, held near one extremity with forceps, is then fused into the end of the rod. Some needles should be perfectly straight, and kept especially for inoculating test-tubes of nutrient jelly. For other purposes the needles may also be bent at the extremity into a small hook or a loop* (Fig. 6).

Tripod Levelling-stand.—A triangular wooden frame supported upon three screw-feet, which

* A looped platinum needle is called in Germany an "öse," a term which, on account of its brevity, may be conveniently adopted

enable it to be raised or lowered to adjust the level.

Large Glass Plate.—A piece of plate-glass, or a pane of ordinary window glass, about a foot square.

Spirit Level.

Glass Bells and Dishes.—Shallow glass bells

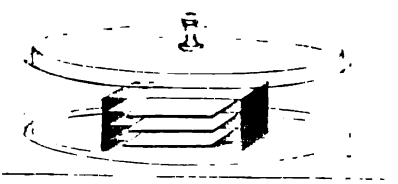


FIG. 7.—DAMP-CHAMBER FOR PLATE CULTIVATIONS.

and dishes, for making a dozen or more damp chambers (Fig. 7), and for completing the ap-

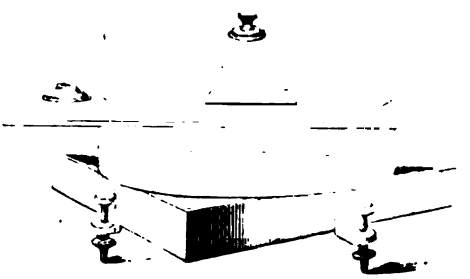


FIG. 8.—APPARATUS EMPLOYED FOR PLATE-CULTIVATIONS.

Tripod stand; Glass dish, filled with cold or iced water; Sheet of Plate-glass; Spirit Level, and Glass Bell.

paratus for pouring out liquefied nutrient jelly on glass plates or slides (Fig. 8).

Iron Box.—A box of sheet-iron (Fig. 9) for

containing glass plates during their sterilisation in the hot-air steriliser, and for storing them until required for use.

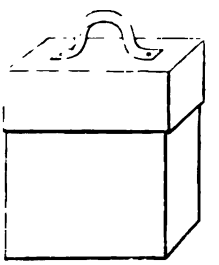


FIG. 9.
BOX FOR GLASS PLATES.

Glass Plates.—Small panes of glass, about six inches by four. Not less than three dozen are required for a dozen damp chambers.

Glass Benches.—These are necessary for arranging the glass plates or slides in tiers in the damp chambers (Fig. 10). Metal shelves may be substituted for them, but the former are to be preferred. They can be easily made, in any number required, by

FIGS. 10, 11.—GLASS BENCHES FOR GLASS PLATES OR SLIDES.

cementing a little piece of plate glass at either end of a glass slip (Fig. 11).

Glass Rods.—One dozen or more glass rods, twelve to eighteen inches in length. They are employed for smoothly spreading out the liquefied nutrient gelatine or agar-agar on the glass plates, etc.

Thermometers. — Two or three centigrade thermometers.

(I) APPARATUS FOR PREPARATION OF POTATO-CULTIVATIONS.

Israel's Case.—Sterilising instruments in the flame of a Bunsen burner is most destructive. It is better, therefore, to have a sheet-iron case (Fig. 12) to contain potato-knives, scalpels, and other instruments, and to sterilise them by placing the case in the hot-air steriliser for an hour at 150° C. The box can be opened at the side, and each instrument withdrawn with a pair of sterilised forceps when required for use.

FIG. 12.—ISRAEL'S CASE.

Glass Dishes.—Several shallow glass dishes are required for preparing damp chambers for potato cultivations (Fig. 13). The upper, being the larger, fits over the lower, and having no handle, admits of these damp chambers being placed, if necessary, in the incubator in tiers. The large size may also be used in the same way for plate cultivations.

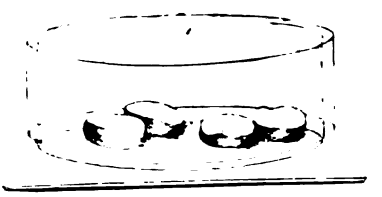


FIG. 13.
DAMP-CHAMBER FOR POTATO-CULTIVATIONS.

Potato Knives.—A common broad smooth-

bladed knife set in a wooden handle is sold for this purpose.

Scalpels. — Half a dozen scalpels, preferably with metal handles, may be kept especially for inoculating sterilised potatoes.

Brush.—A common stout nail-brush, or small scrubbing brush, is essential for cleansing potatoes.

(J) APPARATUS FOR PREPARATION OF SOLIDIFIED STERILE BLOOD SERUM.

Glass Jar.—A tall cylindrical glass jar, on foot, with a broad ground stopper, for receiving blood.

Pipette.—An ordinary or graduated pipette for transferring the serum from the jars to sterile test-tubes or glass capsules.

Serum-Steriliser.—A cylindrical case, with double walls forming an interspace to contain water, closed with a lid, also double walled and provided with a tubular prolongation of the enclosed water chamber (Fig. 14). The water in the cylinder is heated from below, and that in the lid by means of the prolongation.

FIG. 14.—SERUM STERILISER

In the centre of the cylinder is a column which communicates with the water chamber of the cylinder, and from it pass four partitions, which serve to support the test-tubes.

In the lid are three openings, one of which communicates with the water chamber in the lid by which the latter is filled, and into which a thermometer is then fixed. In the centre an opening admits a thermometer, which passes into the central pipe of the cylinder; through a third opening a thermometer passes to the cavity of the cylinder. The cylinder and cover are jacketed with felt, and the apparatus is supported on iron legs.

Serum Inspissator.—A shallow tin case with glass cover, both case and cover jacketed with felt (Fig. 15). The case is double walled, and the water contained in the interspace is heated from below. It is supported on four legs, and the two front ones move in grooves in the case, so that the latter can be placed obliquely at the angle required, and secured in position by screw-clamps. It is employed for coagulating sterile liquid serum, and for solidifying nutrient agar-agar so as to give them a sloping surface.

FIG. 15.—SERUM INSPISSATOR.

Glass Capsules.—Small capsules or hollowed-out cubes of crystal glass are employed for cultivations on solid blood serum, on nutrient gelatine, and on agar-agar. They may be procured of white and blackened glass, and are provided with glass slips as covers.

(K) APPARATUS FOR STORING, AND FOR CULTIVATIONS IN, LIQUID MEDIA.

Lister's Flasks.—Professor Lister devised a globe-shaped flask with two necks; a vertical and a lateral one. The lateral one is a bent spout, tapering towards its constricted extremity. When the vessel is restored to the erect position after pouring out some of its contents, a drop of liquid remains behind in the end of the nozzle, and prevents the regurgitation of air through the spout. A cap of cotton wool is tied over the orifice, and the residue in the flask kept for future use. The vertical neck of the flask is plugged with sterilised cotton wool in the ordinary way.

Sternberg's Bulbs.—Professor Sternberg, of America, advocates the use of a glass bulb, provided with a slender neck drawn out to a fine point and hermetically sealed.*

Aitken's Test-tube.—This is an ingenious device for counteracting the danger of entrance of

* Magnin and Sternberg, *Bacteria*. 1884.

atmospheric germs on removal from the ordinary test-tube of the cotton wool plug. Each test-tube is provided with a lateral arm tapering to a fine point, which is hermetically sealed.

Drop-culture Slides.—About a dozen or more thick glass slides with a circular excavation in the centre are required for drop-cultures.

Vaseline.—A small pot of vaseline with a camel's hair brush should be reserved especially for use in the preparation of drop-cultures.

Bulbed Tubes.—Glass vessels such as test-tubes, flasks, and pipettes, which are used in dealing with liquid media, have already been mentioned under other headings, but bulbed tubes, Pasteur's bulbs, and various other forms are also required for special experiments.

(L) APPARATUS FOR INCUBATION.

There are several forms of incubator, each of which has its advocates. They are mostly rectangular chests, with glass walls, front and back, or in front only. A cylindrical model is preferred by some. Two only will be described here, D'Arsonval's and Babes'. The former admits of very exact regulation of temperature, and the latter is a very practical form for general use.

D'Arsonval's Incubator.—The "*Étuve D'Arsonval*" (Fig. 16) is a very efficient apparatus, and is provided with a heat regulator, which

enables the temperature to be maintained with a minimum variation. It consists of a cylindrical copper vessel, with double walls, enclosing a wide interspace for containing a large volume of water. The roof of the water-chamber is oblique, so that the wall rises higher on one side than on the other. This admits of the interspace being completely filled with water. At the highest point is an opening fitted with a perforated caoutchouc stopper, through which a glass tube passes. The mouth of the cylinder itself is horizontal, and is closed by a lid, which is also double-walled to contain water. In the lid are four openings; one serves for filling its water-chamber, and the others for thermometers and for regulating the air supply in the cavity of the cylinder. The cylinder is continued below by a cone, also double-walled, and there is a perforated grating at

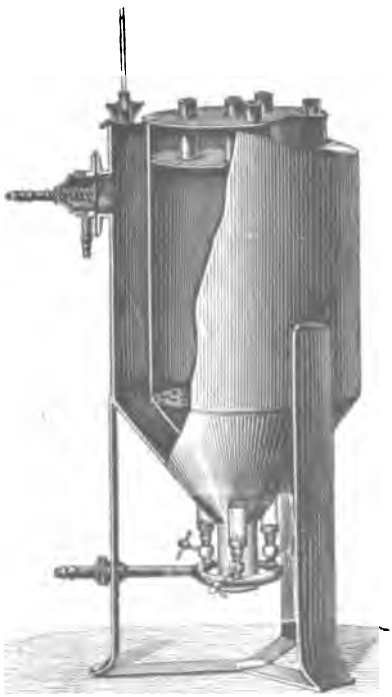


FIG. 16.—D'ARSONVAL'S INCUBATOR.

the line of junction of the cylinder and cone. The cone terminates in a projecting tube provided with an adjustable ventilator. The apparatus is fixed on three supports united to one another below. One of them is utilised for adjusting the height of the heating apparatus. Situated above this leg is the heat regulating apparatus (Fig. 17), attached to a circular, lipped aperture in the outer wall of the incubator. To the lip is fixed with six screws the corresponding lip of a brass box, with a tightly stretched diaphragm of india-rubber intervening. Thus the diaphragm separates the cavity of the box from the water in the interspace of the incubator. The cap of the box, which screws on, is bored in the centre for the screw-pipe, by which the gas is supplied. Another pipe entering the box from below is connected with the gas burners. Around the end of the screw-pipe a collar loosely fits, and is pressed against the diaphragm by means of a spiral wire spring. Close to the mouth of the screw-pipe a small opening exists, so that the gas supply to the burners is not entirely cut off even when the diaphragm completely occludes the mouth of the screw-pipe.

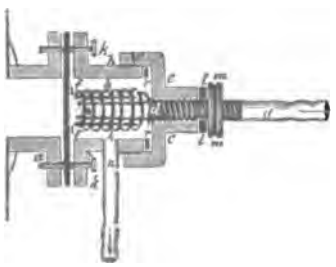


FIG. 17.—SCHLOSING'S MEMBRANE REGULATOR.

To work the apparatus the tube and plug must be

removed, and the water-chamber filled completely with distilled or rain water at the temperature required. The caoutchouc plug is replaced and the tube placed in position. Gas enters through *d* (Fig. 17), and passes through the opening at its extremity into the chamber of the box. Thence it passes through the vertical exit which is connected with the gas burners. As the temperature rises the water rises in the tube, and at the same time exercises a pressure on every part of the walls of the incubator, and hence on the diaphragm. In consequence of this, the diaphragm bulging outwards approaches the end of the tube *d*, and gradually diminishes the gas supply. As a result the temperature falls, the water contracts and sinks in the tube, and the diaphragm receding from *d*, the gas supply is again increased. By adjusting the position of the tube *d* to the diaphragm, any required temperature within the limits of the working of the apparatus can be regulated to the tenth of a degree; *provided*, (1) that the gas supply is rendered independent of fluctuations of pressure, by means of a gas-pressure regulator, (2) that the height of the water in the tube is controlled daily by the withdrawal or addition of a few drops of distilled water, and (3) that the apparatus is kept in a place with as even a temperature as possible, and sheltered from currents of air.

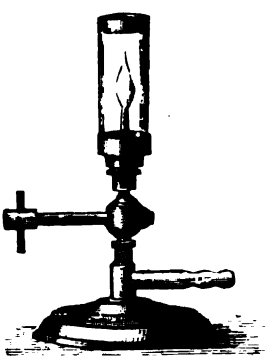


FIG. 18.
GAS BURNER PROTECTED WITH MICA CYLINDER.

The burners in Fig. 16 are protected with mica cylinders similar to the burner represented in Fig. 18. The flames of these burners can be turned down to the smallest length without danger of extinction, and the temperature may be regulated very satisfactorily without using the heat regulator just described, if the gas first

passes through a pressure regulator (Fig. 19). To provide against the danger resulting from accidental extinction of the gas, Professor Koch has devised a self-acting apparatus (Fig. 20), which, simultaneously with the extinction of the flame of the burner, shuts off the supply of gas.

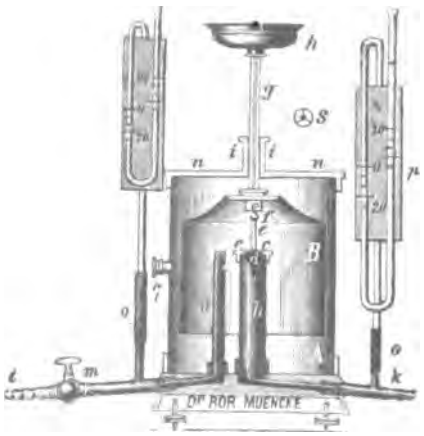


FIG. 19.
MOITESSIER'S GAS-PRESSURE REGULATOR.

Babes' Incubator.—The pattern of Dr. Babes is very simple, and is recommended by the author in preference to all others (Fig. 21).

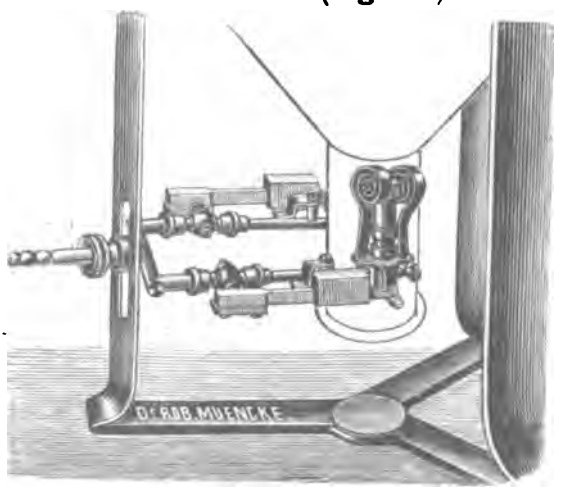


FIG. 20.—KOCH'S SAFETY BURNER.

It consists of a double-walled chest with sides

and roof jacketed with felt. Water fills the interspace between the walls, and on the roof are two apertures, one for a gas regulator, and the other for a thermometer. In front, the chest is closed in by a sheet of felt, a glass door, and a sliding glass panel. The apparatus can be suspended on the wall or supported on legs, and is heated from below by means of protected burners.

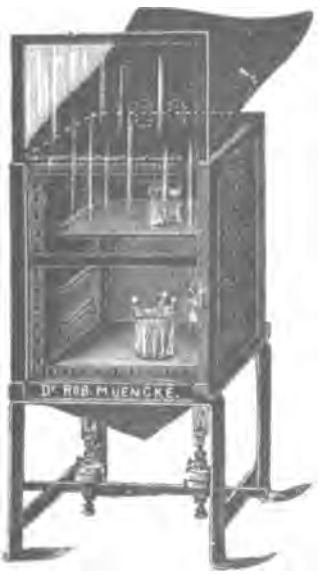


FIG. 21.—BABES' INCUBATOR.

The gas should pass first through a pressure-regulator, and then through a thermo-regulator to the burners.

Moitessier's Gas-pressure Regulator.—This apparatus is best explained by reference to the diagram (Fig. 19). In the bottom of the cylinder A are the entrance (*k*) and exit (*l*) gas tubes. The tap (*m*) regulates the size of the flame. The cover (*n n*) roofs in the cylinder A. The bell (B) supports by means of *e* and *f* the ball valve *d*, which lies in the cover *c c*. The gas, entering by *k*, passes through the valve *d*, and is thence conducted by the tube *a* to the tube *l*. The bell B and the weighted dish *h* are screwed on to the connecting rod *g*. To diminish as much as possible the friction of *g* in

i, *g* only touches *i* by three projecting ridges. Section of *i* and *g* is shown at *s*. To put the apparatus in use it is first levelled, then *h* is screwed off and the cover *n n* removed. A mixture of two parts of pure acid-free glycerine to one of distilled water is poured into the cylinder until it flows out at *q*, which is then closed, and the cover *n n* replaced. The manometers are filled with coloured water, and *k* and *l* connected with the entrance and exit gas tubing respectively. The pressure of the incoming gas raises the bell B; and with it the valve *d* is raised towards the opening at *c c*. The weight *h*, which is replaced on *g*, by its downward pressure counteracts this upward pressure of the gas and opens the valve *c c*. Thus the flame is best regulated in the morning, when the pressure is at a minimum; then supposing an increase of pressure occurs, the weight of *h* is overbalanced, B is raised and with it *d*, and the gas supply proportionately diminished by the gradual closing of the valved opening.

Reichert's Thermo-Regulator.—This regulator (Fig. 22) consists of three parts—a hollow T piece, a stem, and a bulb. The T piece fits like a stopper in the upper widened portion of the stem. One arm of the T is open, and connected with the gas supply; the vertical portion terminates in a small orifice, and is also provided with a minute lateral opening. The stem is provided with a lateral arm, and this arm, the stem, and the bulb

contain mercury. The regulator is fixed in the roof of the incubator, so that the bulb projects either into the interior of the incubator or into the water chamber. When the incubator reaches the required temperature, the mercury is forced up by means of the screw in the lateral arm, until it closes the orifice, at the extremity of the vertical portion of the T. The gas which passes through the lateral orifice is sufficient to maintain the apparatus at the required temperature. If the temperature of the incubator falls the mercury contracts, and gas passing through the terminal orifice of the T, increases the flame of the burner and the temperature is restored.

FIG. 22. REICHERT'S THERMO-REGULATOR.

Page's Thermo-Regulator resembles the above, but instead of the T piece there are two pieces of glass tubing. The outer tubing envelopes the upper part of the stem of the regulator, and admits of being raised or lowered. The upper end of this tubing is closed by a cork, which is perforated to admit the narrow glass tubing, which represents the vertical arm of the T passing within the stem of the regulator. This has a terminal and a lateral opening, and is the means of entrance for the gas. This regulator is adjusted by noting when the thermometer indicates the desired temperature, and then pushing down the outer tube until the terminal

opening of the inner tube, which is carried down with it, is obstructed by the mercury.

Meyer's Thermo-Regulator is represented in Fig. 23. No. I. shows the construction of the

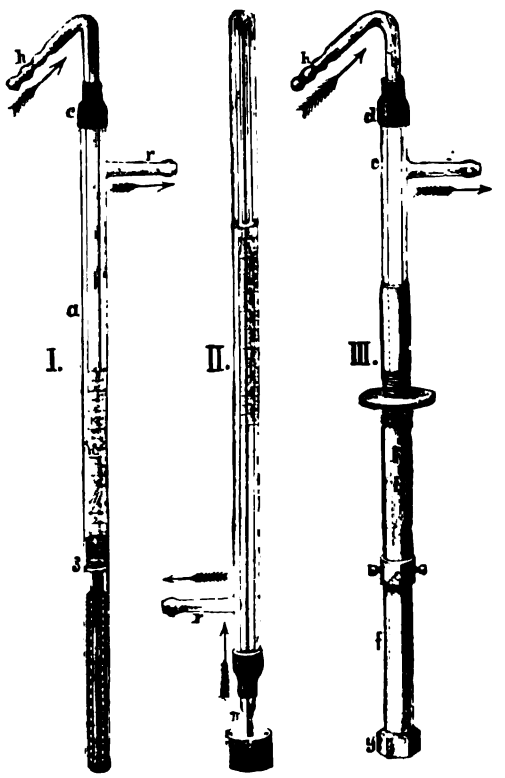


FIG. 23.—MEYER'S THERMO-REGULATOR.

regulator; its inner tube terminates in an oblique opening, and is also provided with a minute lateral aperture, which prevents the complete shutting off of the gas supply. No. II. illustrates

the method of introducing the mercury by suction through a filling tube, which is substituted for the inner tube of the regulator. No. III. represents Fränkel's modification of the same instrument.

(M) INOCULATING AND DISSECTING INSTRUMENTS AND APPARATUS IN COMMON USE.

Mouse Cages.—As mice are the animals most frequently employed for experimental purposes, mouse cages have been especially introduced, consisting simply of a cylindrical glass jar with a weighted wire cover.

Dressing-case.—A small surgical dressing-case, with its usual accessories—forceps, knives, small straight and curved scissors, needles, silk, and so forth—will serve for most purposes.

Pravaz' Syringe.—Koch's modification of Pravaz' syringe admits of sterilisation by exposure to 150° C. for a couple of hours.

Special Instruments and Material.—Instruments required for special operations and the materials necessary for strict antiseptic precautions need not be detailed here.*

Dissecting Boards.—Slabs of wood in various sizes, or gutta percha trays, provided with large-headed pins, are employed for ordinary purposes.

Dissecting Case.—A dissecting case fitted with scalpels, scissors, hooks, etc., should be reserved entirely for post-mortem examinations.

* *Vide* Cheyne, *Antiseptic Surgery*. 1882.

(N) GENERAL LABORATORY REQUISITES.

Siphon Apparatus.—Two half-gallon or gallon glass bottles, with siphons connected with long flexible tubes provided with glass nozzles and pinchcocks (Fig. 24), should be employed for the

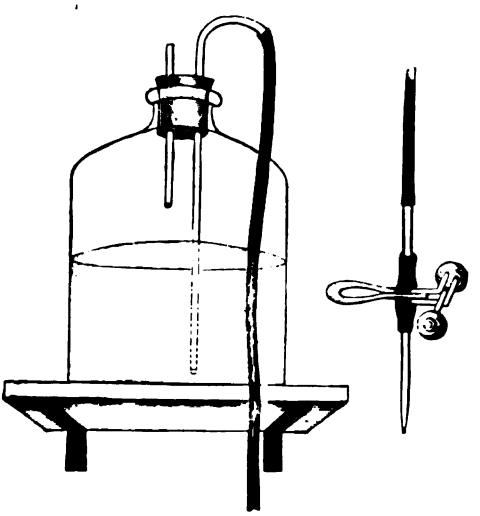


FIG. 24.
SIPHON BOTTLE, WITH FLEXIBLE TUBE, GLASS NOZZLE, AND A MOHR'S PINCHCOCK.

following purposes :—One is used to contain distilled water, with the nozzle hanging down conveniently within reach of the working table; the other is to contain a solution of corrosive sublimate (1 in 1000), and may be placed so that the nozzle hangs close to the lavatory sink or basin. The former replaces the use of the ordinary wash bottle, in washing off

surplus stain from cover glasses, etc., and the latter is conveniently placed for disinfection of vessels and hands after cleansing with water. They should be placed on the top of a cupboard, or on a high shelf.

Desiccator—The Desiccator (Fig. 25) consists of a porcelain pan containing concentrated sulphuric acid, and covered over with a bell-glass receiver.

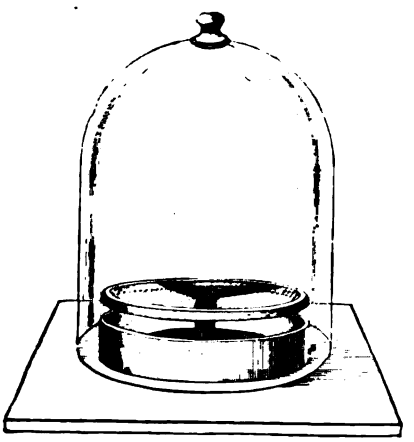


FIG. 25.—DESICCATOR.

The sheet of plate-glass upon which the pan rests is ground upon its upper surface, and the rim of the glass bell is also ground and well greased. In the centre of the pan is a column supporting a circular frame, which is covered with wire gauze. Slices of potatoes, upon which micro-organisms have been cultivated, are rapidly dried by the action of the sulphuric acid in confined air. A cultivation of *Bacterium prodigiosum*, for example,

may be dried in this way, and preserved for subsequent experiments.

Other items commonly in use in a research laboratory cannot be detailed here, and a description of air-pumps, refrigerators, etc., access to which is nevertheless necessary for some special investigations, must be sought for elsewhere.*

* All bacteriological apparatus, as employed by Professor Koch, may be obtained from Dr. Muencke, 58, Louisen Strasse, Berlin. Nearly all the figures of apparatus here given are from blocks, kindly lent to me by Dr. Muencke. Griffin & Son, 22, Garrick Street, Covent Garden, W.C., will make *to order* any bacteriological apparatus required, and from them all glass vessels and chemical apparatus of home manufacture can be obtained. All histological instruments and material, such as microscopes, microtomes, aniline dyes, celloidin, gelatine, agar-agar, etc., are supplied by G. Kõnig, Berlin, N.W., 35, Dorotheen Strasse. Chemicals, staining reagents, and ready-prepared nutrient gelatine can also be obtained from Dr. Georg Grübler, Leipsig, 17, Dufour Strasse. Solutions of lithium-carmine, picro-lithium carmine (Orth.), picro-carmine (Weigert), alum and borax-carmine (Grenacher), etc., ready for use, are prepared by Becker & Co., 34, Maiden Lane, Covent Garden, London, W.C. The latter firm also keep in stock bacteriological apparatus and glass ware of the German pattern.

Mr. Baker, of High Holborn, W.C., is recommended for the supply of microscopes and the ordinary objectives by continental makers, and the new apochromatic objectives recently introduced by Zeiss. Objectives made of the new glass are also constructed by Powell and Leland, but though invaluable to the specialist their expense places them beyond the reach of the general student. Messrs. Swift & Son have recently introduced an excellent $\frac{1}{12}$ oil. imm. for five guineas, and are prepared to supply a microscope completely equipped for bacteriological work at a very low price.

CHAPTER III.

MICROSCOPICAL EXAMINATION OF BACTERIA IN LIQUIDS, IN CULTIVATIONS ON SOLID MEDIA, AND IN TISSUES.

Preliminary Remarks. — In conducting bacteriological researches, the importance of absolute cleanliness cannot be too strongly insisted upon. All instruments, glass vessels, slides, and cover glasses should be thoroughly cleansed before use. A wide-mouthed glass jar should always be close at hand, containing refuse alcohol for the reception of rejected slide preparations, or dirty cover-glasses. When required again for use, slides can be easily wiped clean with a soft rag. Cover-glasses require further treatment, for unless they are perfectly clean it is difficult to avoid the presence of air bubbles when mounting specimens. They should be left in strong acid (hydrochloric, sulphuric, or nitric) for some hours; they are then washed, first with water and then with alcohol, and carefully wiped with a soft rag. The same principle applies in the preparation and employments of culture media; any laxity in the processes of sterilisation, or insufficient atten-

tion to minute technical details, will surely be followed with disappointing results in the contamination of one's cultures, resulting in the loss of much time. When using platinum needles, either for inoculating fresh tubes in carrying on a series of pure cultures, or in transferring a small portion of a cultivation to a cover-glass for examination under the microscope, the careful sterilisation of the needle by heating the platinum wire till it is white hot in every part, and heating also as much of the glass rod as is made to enter the test-tube, must be carried out with scrupulous care. Indeed it is a good plan to let it become a *force of habit* to sterilise the needle before and after use on every occasion, whatever may be the purposes for which it is employed.

(A) EXAMINATION IN THE FRESH STATE.

Liquids containing micro-organisms such as pus, blood, juices, culture-fluids, can be investigated by transferring a drop with a sterilised öse or a capillary pipette to a slide, covering it with a clean cover-glass, and examining without further treatment. If it is desirable to keep the specimen under prolonged observation, a drop of sterilised water or salt solution must be run in at the margin of the cover-glass to counteract the tendency to dry. *Cultures on solid media* can be examined by transferring a small portion with a

sterilised needle to a drop of sterilised water on a slide, thinning it out, and covering with a cover-glass as already described. A more satisfactory method, by which one can keep micro-organisms under observation and study their movements, spore-formation, etc., will be described under "Drop-cultures." *Tissues in the fresh state* may be teased out with needles in sterilised salt solution, and pressed out into a sufficiently thin layer between the slide and cover-glass. Glycerine may in many cases be substituted for salt solution, especially for the examination of micro-organisms such as Actinomyces, Aspergilli, etc.

There is as a rule no difficulty in recognising the larger micro-organisms such as those just mentioned, but where we have to deal with very small bacilli, bacteria and micrococci, they may possibly be mistaken for granular detritus or fat-crystals, or *vice-versâ*. They are distinguished by the fact that fatty and albuminous granules are altered or dispersed by acetic acid, and changed by solution of potash; alcohol, chloroform, and ether dissolve out fat crystals or fatty particles; on the other hand, micro-organisms remain unaffected by these re-agents. This micro-chemical reaction is made the basis of Baumgarten's method (p. 278).

(B) COVER-GLASS-PREPARATIONS.

The method next to be described is the most commonly employed; in addition to its value as a means of examining liquids, etc., it affords the additional advantage of enabling one to make, if necessary, a large number of preparations which when dried can be preserved, stained or unstained, in ordinary cover-glass boxes; they are then in a convenient form for transport, and can be mounted permanently at leisure.

The method is as follows:—A cover-glass is smeared with the cut surface of an organ, or pathological growth, or with sputum; or a drop of blood, pus, or other fluid to be examined, is conveyed to it with a large öse. By means of another cover-glass, the juice, or fluid, is squeezed out between them into a thin layer, and on sliding them apart each cover-glass bears on one side a thin film of the material to be examined. They are then placed with the prepared side upwards and allowed to dry. After a few minutes, they are held with a pair of flat-bladed or spring forceps, with the prepared side uppermost, and passed rapidly three times through the flame of a spirit lamp or Bunsen burner. To stain them, put two or three drops of an aqueous solution of fuchsine or methyl violet over the film, and after a minute or two wash off the surplus stain with distilled water by means of the siphon apparatus or a wash

bottle. Turn the cover-glass on to a slide, remove excess of water with filter paper, and wipe the exposed surface; examine with Zeiss' DD (about 230 diams.), and if a higher power be required, which is usually the case, place a droplet of cedar oil on the cover glass, and examine with an immersion lens.

If the specimen is to be made permanent, fix the cover-glass at one corner with the thumb, and with a soft rag carefully wipe off the cedar oil; then float off the cover-glass by running in distilled water at its margin, and having made a little ledge with a strip of filter-paper, place the cover-glass up against it upon one of its edges and leave it to dry. When perfectly dry mount in Canada balsam, or put it away in a cover-glass box provided with a label of contents.

A culture from a solid medium may be stained and examined in the same way after spreading it out with a needle into a thin film, with or without the addition of a droplet of sterilised water.

In many cases it is necessary or preferable to apply the stain for a much longer period. This is effected by pouring some of the staining solution into a watch-glass, and allowing the cover-glasses to swim on the surface, with their prepared side, of course, downwards. Throughout all these manipulations it is necessary to bear in mind which is the prepared surface of the cover-glass.

Double coloration of cover-glass prepara-

tions can also be obtained as in Ehrlich's method for staining tubercular sputum, or by staining with eosin after treatment by the method of Gram.

Ehrlich's Method is as follows :—Five parts of aniline oil are shaken up with one hundred parts of distilled water, and the emulsion filtered through moistened filter paper. A saturated alcoholic solution of fuchsine, methyl violet, or gentian violet, is added to the filtrate in a watch-glass drop by drop until precipitation commences. Cover-glass preparations are floated in this mixture for fifteen minutes to half an hour, then washed for a few seconds in diluted nitric acid (one part nitric acid to two of water) and then rinsed in distilled water. The stain is removed from everything except the bacilli, but the ground substance can be after-stained, brown if the bacilli are violet, or blue if they have been stained red (Plate XX., Fig. 1).

Double staining with eosin after the method of Gram is described under tissue staining. The cover-glass preparations are treated by the same processes as employed with sections ; superfluous oil of cloves can be removed by gently pressing the cover-glass between double layers of filter paper.

Babes' Method affords a very rapid means of examining cultivations, etc. A little of the growth, removed by means of a sterilised platinum hook or small öse, is spread out on a cover glass into as thin a film as possible : when almost dry, a drop or two of a weak aqueous solution of methyl violet is

allowed to fall from a pipette upon the film. The cover-glass with the drop of stain is after a minute carefully turned over on to a slide, and the excess of stain gently and gradually removed by pressure with a strip of filter paper. It affords a rapid means of demonstration, for example of such a cultivation as Koch's comma bacilli in nutrient gelatine, enabling the microbes to be seen in some parts of the preparation both stained and in active movement.

His' Method.—The staining of fresh preparations, especially those with no coagulable albumen to fix them, may be also carried out by His' method. A slide is prepared as already described in the examination of micro-organisms in the fresh state. The reagents are then applied by placing them with a pipette drop by drop at one margin of the cover-glass, and causing them to flow through the preparation by means of a strip of filter paper placed at the opposite margin.

To stain spores the method described on p. 48 is somewhat modified. The cover-glass preparations may be either passed as many as twelve times through the flame, or heated to a temperature of 210° for half-an-hour, or exposed to the action of strong sulphuric acid for a few seconds, and then stained with a watery solution of the dye.

To double-stain spore-bearing bacilli.—The cover-glass preparations may be floated for twenty minutes on a fuchsine aniline-water solution, as

used in Ehrlich's method, which has been heated to boiling-point. The fuchsine is removed from the bacilli either by simply rinsing in water, in alcohol, or in weak acid, according to the species, and then the preparations are floated for a few minutes on solution of methylene blue, rinsed in water, dried and mounted.

To stain flagella.—Koch recommends floating the cover-glasses on a concentrated watery solution of hæmatoxylin. From this they are transferred to a 5 per cent. solution of chromic acid or to Müller's fluid, by which the flagella obtain a brownish-black coloration. The author has succeeded in demonstrating and photographing flagella, by staining with a drop of a saturated solution of gentian violet in absolute alcohol. Before the alcohol has time to evaporate the cover-glass is rinsed in water, and then allowed to dry, and finally mounted in balsam. A very intense staining of the whole preparation results.

(c) COVER-GLASS IMPRESSIONS.

One of the most instructive methods for examining micro-organisms is to make what is called in German a "*Klatsch Präparat.*" It enables us in many cases to study the relative position of individual micro-organisms one to another in their growth on solid cultivating media, and in some cases produces the most exquisite preparations for

the microscope. A perfectly clean, usually small-sized, cover-glass is carefully deposited on a plate or potato culture, and gently and evenly pressed down. One edge is then levered up, carefully, with a needle and the cover-glass lifted off by means of forceps. It is then allowed to dry, passed through the flame three times, and stained as already described. In the case of plate-cultures, especially where no liquefaction has taken place, the growth is bodily transferred to the cover-glass and a vacant area left on the gelatine or agar-agar, corresponding exactly with the form and size of the cover-glass employed (Plate XXV., Figs. 1 and 2).

CHAPTER IV.

PREPARATION AND STAINING OF TISSUE SECTIONS.

(A) METHODS OF HARDENING AND DECALCIFYING PREPARATIONS.

To harden small organs, such as the viscera of a mouse, they must be placed on a piece of filter paper at the bottom of a small, wide-mouthed glass jar, and covered with about twenty times their volume of absolute alcohol. Larger organs, pathological growths, etc., are treated in the same way, but must first be cut into small pieces, or cubes, varying from a quarter of an inch to an inch in size. Muller's fluid may also be employed, and methylated spirit may be substituted for alcohol, from motives of economy. Tissues hardened in absolute alcohol are ready for cutting in two or three days, and those hardened in Muller's fluid in as many weeks.

Teeth, or osseous structures, must first be placed in a decalcifying solution, such as Kleinenberg's. When sufficiently softened they are allowed to soak

in water, to wash out the picric acid, and then transferred through weak spirit to absolute alcohol. Ebner's solution also gives excellent results, especially when the structures to be decalcified are placed in fresh solution from time to time.

(B) METHODS OF EMBEDDING, FIXING, AND CUTTING.

Material to be cut with the freezing microtome, if hardened in spirit, must be well soaked in water before being frozen; if hardened in Muller's fluid, it can be frozen at once.

If Williams' microtome is employed, the hardened tissues must first be well soaked in gum mucilage, then frozen, and cut.

For cutting with Jung's microtome, the tissues are embedded in paraffin, or celloidin, and mounted on cork, or, if firm enough, they may be fixed upon cork without any embedding material at all. Paraffin, dissolved in chloroform, will be found very serviceable as an embedding material, but celloidin is more commonly employed now. The pieces of tissue to be embedded are placed, after the process of hardening is completed, in a mixture of ether and alcohol for an hour or more. They are then transferred to a solution of celloidin in equal parts of ether and alcohol, and left there, usually, for several hours. Meanwhile, corks ready cut for the clamp of the microtome are smeared

over with the solution of celloidin ; this is applied with a glass rod to the surface which is to receive the piece of tissue. The corks are then set aside for the film of celloidin to harden. The pieces of tissue are allowed to remain in the celloidin solution for from one to twenty-four hours, the time varying according to the structure of the specimen. Better results are obtained in the case of lung, or degenerated broken-down tissue, if left for a much longer time than is found to be sufficient for firmer structures. The specimen, when ready, is removed from the celloidin solution with forceps, and placed upon a prepared cork. A little of the solution, which is of syrupy consistence, is allowed to fall on the piece of tissue to cover it completely, and the mounted specimen is finally placed in 60 to 80 per cent. alcohol to harden the celloidin. The specimen will be ready for cutting next day.

The specimen may be more neatly embedded by fixing it with a pin in a small paper tray, pouring the celloidin solution over it, and then placing the tray in alcohol to harden the celloidin. The embedded specimen is then fixed on a cork, which has been cut for the clamp of the microtome. The celloidin in the section disappears in the process of clearing with clove-oil.

Material infiltrated with paraffin must be cut perfectly dry, and the sections prevented from rolling up by gentle manipulation with a camel's-hair brush. They must then be picked off the blade of

the knife with a clean needle, and dropped into a watch-glass containing xylol. This dissolves out the paraffin; the sections are then transferred to alcohol to get rid of the xylol, and then to the staining solution.

In the case of specimens embedded in celloidin, or mounted directly on a cork, the tissue, as well as the blade of the knife, should be kept constantly bathed with alcohol, and the sections transferred from the blade with a camel's hair brush, and floated in alcohol.

For fixing small organs and pieces of firm tissue directly on cork, such as the kidneys of a mouse, or liver, one employs gelatine, or glycerine-gelatine, liquefied over a Bunsen burner in a porcelain capsule. The cork with specimen affixed is placed in alcohol, and is ready for cutting sections next day.

The advantage of glycerine-gelatine consists in that it may be used for fixing irregular pieces of tissue, as it does not become of a consistency that would injure the edge of the knife.

(c) GENERAL PRINCIPLES OF STAINING BACTERIA IN TISSUE SECTIONS: METHODS OF WEIGERT, GRAM, AND WEIGERT-EHRLICH.

Sections of fresh tissues made with the freezing microtome are to be floated and well spread out in ·8 per cent. salt solution, and then carefully transferred, well spread out on the copper lifter, to a watch-glass containing absolute alcohol. Simi-

larly sections selected from those cut with Jung's microtome, may be transferred from the spirit to absolute alcohol. The sections may be then stained by any of the methods to be described.

It is often advisable to employ some method which will enable one to study the structure of the tissue itself. In the same way with sections however prepared, one should always examine with a low power (Zeiss' AA) first; this enables one to recognise the tissue under examination in most cases, and even to examine in many cases the topographical distribution of masses of bacteria. With Zeiss' DD., Oc. 2, a power of about 250 diams., very many bacteria can be distinguished, and with the oil immersion lenses the minutest bacilli and micrococci can be recognised, and the exact form of individual bacteria accurately determined. As Zeiss' microscopes are, like most good modern instruments, provided with a triple nosepiece, there is no loss of time in examining a preparation successively with these different powers.

Weigert's Method.—A very useful method for staining both the tissue and the bacteria is as follows:—Place the sections for from six to eighteen hours in a one per cent. watery solution of any of the basic-aniline dyes (methyl violet, gentian violet, fuchsine, bismarck brown). To hasten the process place the capsule containing the solution in the incubator, or heat it to 45° C. A stronger solution may also be employed, in which case the sections

are far more rapidly stained, and are easily overstained. In the latter case they must be treated with a half-saturated solution of carbonate of potash. In either case the sections are next washed with distilled water, and passed through 60 per cent. alcohol into absolute alcohol. When almost decolorised spread out the section carefully on a copper lifter and transfer it to clove-oil, or stain with picro-carmine solution (Weigert's) for half-an-hour, wash in water, alcohol, and then treat with clove-oil. After the final treatment with clove-oil, transfer with the copper lifter to a clean glass slide. Dry the preparation by pressure with a piece of filter paper folded four times, and preserve in Canada balsam dissolved in xylol.

Gram's Method.—In the method of Gram the sections are stained for three minutes in aniline-gentian-violet solution. This is prepared by shaking up one ccm. of pure aniline with twenty four parts of water, and filtering the emulsion. Half a gramme of the best finely powdered gentian violet is dissolved in the clear filtrate, and the solution filtered before use. The sections are then transferred to a solution of iodine in iodide of potassium till they become dark brown in colour, and then decolorised in absolute alcohol. The time required for complete decolorisation in alcohol varies from a few minutes to twenty-four hours. They are then treated with clove-oil and mounted in Canada balsam. It is much better, however, to employ

the aniline-gentian-violet solution quite freshly prepared, and the following useful method is invariably used by the author:—Place four or five drops of pure aniline in a test-tube, fill it three-quarters full with distilled water, close the mouth of the tube with the thumb and shake it up thoroughly. Filter the emulsion twice, and pour the filtrate into a watch-glass or glass capsule. To the perfectly clear aniline water thus obtained add drop by drop a concentrated alcoholic solution of gentian-violet till precipitation commences. Stain sections in this solution from ten minutes to half an hour, then transfer to iodine-potassic-iodide solution, and decolorise in alcohol. The process of decolorisation may be hastened by placing the section in clove-oil and returning it to alcohol, and again to clove-oil. If examined, after it has been finally treated with clove-oil and mounted in Canada balsam, the tissue appears colourless or tinged faintly yellow, while micro-organisms, *e.g.*, bacilli and micrococci, are stained blue or blue-black. Double staining is obtained by transferring the sections after decolorisation to a solution of eosin, bismarck brown, or vesuvin, again rinsing in alcohol, clearing in clove-oil, and mounting in balsam. Another instructive method is to place the decolorised sections in picro-carminate of ammonia for three or four minutes, and then treat with alcohol, oil of cloves, and balsam. In this way the nuclei are well stained. A somewhat similar

result is obtained by placing the sections for a few minutes in Orth's solution (picro-lithium-carmine); transferring to acidulated alcohol, then to an alcoholic solution of picric acid, and treating with clove-oil and balsam (Plate XVII., Fig. 1).

Weigert-Ehrlich Method.—This is a method in which nitric acid is employed as a decolorising agent. It is as follows:—Filtered, saturated, watery solution of aniline one hundred parts; saturated alcoholic solution of a basic aniline dye (methyl-violet, gentian-violet, fuchsine), eleven parts; are mixed and filtered. Rapid staining is obtained by warming the solution. Decolorise with nitric acid (1 in 2), and stain with a contrast colour. As delicate sections are apt to be injured by immersion in the nitric acid, they may be transferred from the fuchsine solution to distilled water, then rinsed a few minutes with alcohol, and finally placed in the following contrast stain for one to two hours :—

Distilled water . . .	100 cc.
Saturated alcoholic solution of methylene blue. . .	20 cc.
Formic acid	10 mm.*

The method of Weigert-Ehrlich is employed for staining both leprosy and tubercle bacilli. The aniline-fuchsine solution may also be prepared by the simple plan described for Gram's method (page 60). The more special methods for staining will be given with the description of those species of micro-organisms to which they apply.

* Watson Cheyne, *Practitioner*. 1883.

CHAPTER V.

PREPARATION OF NUTRIENT MEDIA AND METHODS OF CULTIVATION.

To cultivate micro-organisms artificially, and, in the case of the pathogenic bacteria, to fulfil the second of Koch's postulates, they must be supplied with nutrient material free from pre-existing micro-organisms. Hitherto various kinds of nutrient liquids have been employed, and in many cases they still continue to be used with advantage, but as a general rule they have been in a great measure supplanted by the methods of cultivation on sterile solid media about to be described. The advantages of the latter method are obvious. In the first place, in the case of liquid media, in spite of elaborate precautions and the expenditure of much labour and time, it was almost impossible or extremely difficult to obtain a pure culture. If a drop of liquid containing several kinds of bacteria be introduced into a liquid medium, we have a mixed cultivation from the very first. If in the struggle for existence some bacteria were unable to develop in the presence of others, or a change of temperature and soil allowed

one form to predominate over another, then one might be led to the conclusion that many bacteria were but developmental forms of one and the same micro-organism; while possibly the contamination of such cultures might lead to the belief in the transformation of a harmless into a pathogenic bacterium. In the case of solid cultivating media, on the other hand, the chance of contamination by gravitation of germs from the air is avoided by the fact that test-tubes, flasks, etc., can be inverted and inoculated from below. The secret of the success of Koch's method, however, depends upon the possibility, in the case of starting with a mixture of micro-organisms, of being able to isolate them completely one from another, and to obtain an absolutely pure growth of each cultivable species. When sterile nutrient gelatine has been liquefied in a tube and inoculated with a mixture of bacteria in such a way that the individual micro-organisms are distributed throughout it, and the liquid is poured out on a plate of glass and allowed to solidify, the individual bacteria, instead of moving about freely as in a liquid medium, are fixed in one spot, where they develop individuals of their own species. In this way colonies are formed each possessing its own characteristic biological and morphological appearances. If an adventitious germ from the air falls upon the culture, it also grows exactly upon the spot upon which it fell, and can be easily recognised as a stranger. To maintain the individuals isolated from one another

during their growth, and free from contamination, it is only necessary to thin out the cultivation, and to protect the plates from the air. The slower growth of the micro-organisms in solid media, and the greater facility afforded thereby for examining them at various intervals and stages of development, is an additional point in favour of these methods; and the characteristic macroscopical appearances so frequently assumed are, more especially in the case of morphological resemblance or identity, of the greatest importance. The colonies on nutrient gelatine (examined with a low power) of *Bacillus anthracis* and *Proteus mirabilis;* the naked eye appearances in test-tubes of nutrient gelatine of the bacillus of mouse-septicæmia (Figs. 114, 115), and of anthrax (Fig. 107), and the brilliant and curious growth of *Bacterium indicum* upon nutrient agar-agar (Plate II., Fig. 1), may be quoted as examples in which the appearances in solid cultivations are pathognomonic.

Solid Media

(A) Preparation of Sterile Gelatine-, and Agar-Agar-Peptone-Broth.

Sterile Gelatine-Peptone-broth, or Nutrient Gelatine, is prepared as follows:—Take half a kilogramme of beef (one pound), as free as possible from fat. Chop it up finely, transfer it to a flask or cylindrical vessel, and shake it up well with a

litre of distilled water. Place the vessel in an ice-pail, ice-cupboard, or in winter in a cold cellar, and leave for the night. Next morning commence with the preparation of all requisite apparatus. Thoroughly wash, rinse with alcohol, and allow to dry, about 100 test-tubes. Plug the mouth of the test-tubes with cotton wool, taking care that the plugs fit firmly, but not too tightly. Place them in their wire cages in the hot-air steriliser to be heated for an hour at a temperature of 150° C. In the same manner cleanse and sterilise several flasks and a small glass funnel. In the meantime the meat infusion must be again well shaken, and the liquid portion separated by filtering and squeezing through a linen cloth. The red juice thus obtained must be brought up to a litre by again transferring it to a large measuring glass and adding distilled water. It is then poured into a sufficiently large and strong beaker; and set aside after the addition of

10 grammes of peptonum siccum.
5 grammes of common salt.
100 grammes of best gelatine.

In about half-an-hour the gelatine is sufficiently softened, and subsequent gently heating in a water-bath causes it to be completely dissolved. The danger of breaking the beaker may be avoided by placing a cloth several times folded at the bottom of the water-bath.

The next process requires the greatest care and attention. Some micro-organisms grow best in a slightly acid, others in a neutral or slightly alkaline medium. For example, for the growth and characteristic appearances of the comma bacillus of Asiatic cholera a faintly alkaline soil is absolutely essential. This slightly alkaline medium will be found to answer best for most micro-organisms, and may be obtained as follows :—

With a clean glass rod dipped into the mixture, the re-action upon litmus paper may be ascertained, and a concentrated solution of carbonate of soda must be added drop by drop, until red litmus paper becomes faintly blue. If it has been made too alkaline it can be neutralised by the addition of lactic acid.

Finally, the mixture is heated for an hour in the water-bath and filtered while hot. For the filtration the hot-water apparatus can be used with advantage, furnished with a filter of Swedish paper made in the following way.

About eighteen inches square of the best and stoutest filtering paper is first folded in the middle, and then, as in Fig. 26, creased into sixteen folds. The filter is made to fit the glass funnel by gathering up the folds like a fan, and cutting off the superfluous part. The creasing of each fold should be made firmly to within half an inch of the apex of the filter, which part is to be gently inserted into the tube of the funnel. To avoid

bursting the filter at the point, the broth when poured out from the flask should be directed against the side of the filter with a glass rod. During filtration the funnel should be covered over with a circular plate of glass, and the process of filtration must be repeated, if necessary, until a pale, straw-coloured, perfectly transparent filtrate results.

The sterilised test-tubes are filled for about a third of their depth by pouring in the gelatine

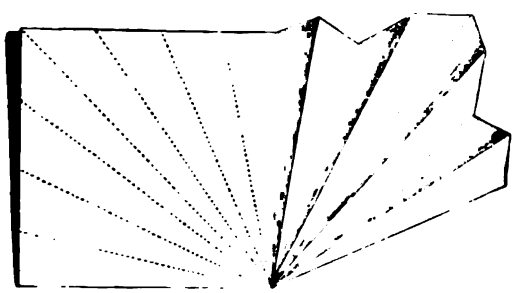


Fig. 26.—METHOD OF MAKING A FOLDED FILTER.

carefully and steadily, or by employing a small sterilised glass funnel. The object of this care is to prevent the mixture touching the part of the tube with which the plug comes into contact; otherwise, when the gelatine sets, the cotton wool adheres to the tube, and becomes a source of embarrassment in subsequent procedures. As the tubes are filled they are placed in the test-tube basket, and must then be sterilised. They are either lowered into the steam steriliser, when the

thermometer indicates 100° C., for twelve minutes for four or five successive days; or they may be transferred to the test-tube water-bath and heated for an hour a day for three successive days.

If the gelatine shows any turbidity after these processes, it must be poured back from the test-tubes into a flask and boiled up for ten minutes, after the addition of the white and shell of an egg beaten up together. It is once more filtered, and the processes of sterilisation just described must be repeated.

Sterile Agar-Agar-Peptone-broth, or Nutrient Agar-Agar.—Agar-Agar has the advantage of remaining solid up to a temperature of about 45°. The preparation of a sterile nutrient jelly is conducted on much the same principles as those already described. Instead, however, of 100 grammes of gelatine, only about 20 grammes of agar-agar are employed (1·5—2 per cent.), and to facilitate its solution it must be allowed to soak in salt-water overnight. For the filtration, flannel is substituted for filter paper, or may be used in combination with the latter. The hot-water apparatus is invariably employed, unless, to accelerate the process, the glass funnel and receiver are bodily transferred to the steam steriliser. If the conical cap cannot be replaced, cloths laid over the mouth of the steriliser must be employed instead. It may be necessary to repeat the process of filtration, but it must not be

expected that such a brilliant transparency can be obtained as with gelatine. The final result, when solid, should be colourless and clear, but if only slightly milky it may still be employed.

After the final treatment in the steam steriliser some of the tubes may be placed in the blood-serum apparatus, and left to gelatinise with an oblique surface. A little liquid gradually collects at the bottom of the surface, being expressed by the contraction of the nutrient jelly.

(B) METHODS OF EMPLOYING NUTRIENT JELLY IN TEST-TUBE- AND PLATE-CULTIVATIONS.

Test-Tube-Cultivations.—To inoculate test-tubes containing nutrient jelly, the cotton wool plug must be *twisted* out, by which means any adhesions that may exist are broken down. A sterilised needle charged with the blood, pus, etc., containing the micro-organisms, or with a colony from a plate-culture, is steadily thrust once, and once only, into the nutrient jelly. The tube should be held with its mouth downwards, to avoid, as far as possible, accidental contamination from the gravitation of germs in the air, and the plug, which has been removed with the thumb and index finger of the right hand and held between the fourth and fifth fingers of the left, is replaced as rapidly as possible (Fig. 27).

The chances of error arising from contamination

of the cultivations are further reduced by avoiding draughts at the time of inoculation, and it is best that these manipulations should be carried on in a quiet room in which the tables and floor are wiped with damp cloths, rather than in a laboratory in which the air becomes charged with germs through constant sweeping and dusting, and the entrance and exit of classes of students. In conducting any investigation a dozen or more tubes should be inoculated, and if by chance an adventitious germ, in spite of these precautions, gain an entrance, the contaminated tube can be rejected and the experiments continued with the remaining pure cultivations.

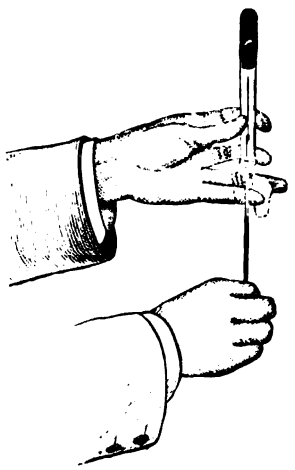


FIG. 27.
METHOD OF INOCULATING A TEST-TUBE CONTAINING STERILE NUTRIENT JELLY.

Where, however, one tube is inoculated from another containing a liquid medium, as in the process of preparing plate-cultures, or where a culture is made from a tube in which the growth has liquefied the gelatine, it is obvious that the tubes cannot be inverted, and they must then be held and inoculated as in Fig. 28. To inoculate those tubes of nutrient agar-agar which have been gelatinised obliquely, the sterilised needle with the material to be cultivated is streaked over the surface from below upwards.

Examination of test-tube cultivations.—The appearances produced by the growths in test-tubes can be in most cases sufficiently examined with the naked eye. The illustrations in the accompanying plates (Plates II., III., IV., V., VIII., XIII., XIV., XXIV.) are given as examples of the various changes produced in the nutrient media. In some cases the jelly is partially or completely liquefied, while in others it remains solid. The growths may be abundant or scanty, coloured or colourless. The nutrient jelly may itself be tinged or stained with products resulting from the growth of the organisms. When liquefaction slowly takes place in the needle track, or the organism grows without producing this change, the appearances which result are often very delicate, and in some cases very characteristic. The appearance of a simple white thread, of a central thread with branching transverse filaments, of a cloudiness, or of a string of beads in the track of the needle, may be given as examples. In such cases much may be learnt by examining the growth with a magnifying glass. Here, however, a difficulty is encountered, for the cylindrical form of the tube so distorts the appearance of its contents, that the examination is rendered somewhat difficult. To obviate this, the following very simple contrivance may be employed with advantage.

Cheshire's Trough.—This consists of a rectangular vessel, four inches in height, two inches in

width, and one inch in depth. It may be easily constructed by cementing together two slips of glass to form the back and front, with three slips of stout glass with ground edges forming the sides and base. The front may be constructed of thin glass, and the base of the vessel made to slope so that the test-tube when placed in the trough has a tendency to be near the front. The trough is filled with a mixture of the same refractive index as the nutrient gelatine. The latter has a refractive index rather higher than water, which is about 1·333; alcohol has a refractive index of 1·374. The trough is filled with water, and alcohol is then added until the proper density is reached. The test-tube is placed in the trough, and held in position by means of a clip. The trough can be fixed on the inclined stage of the microscope, and the contents of the tube conveniently examined with low power objectives.

PLATE-CULTIVATIONS.

The key to the success of Koch's method of cultivation on solid media consists in the employment of plate-cultivations. By this means, as has already been mentioned (p. 63), a mixture of bacteria, whether it be in fluids, excreta, or in artificial cultivations, can be so treated that the different species are isolated one from the other, and perfectly pure cultivations of each of the cultivable bacteria in the original mixture established in various nutrient media. We are enabled

also to examine under a low power of the microscope the individual colonies of bacteria, and to distinguish, by their characteristic appearances, micro-organisms which, in their individual form, closely resemble one another, or are even identical. The same process, with slight modification, is also employed in the examination of air, soil, and water, as will be referred to later.

The preparation of plate-cultivations, therefore, must be described in every detail, and to take an example, we will suppose that a series of plates are to be prepared from a test-tube-cultivation.

Arrangement of Levelling Apparatus.—In order to spread out the liquid jelly evenly on the surface of a glass plate, and hasten its solidification, it is necessary to place the glass plate upon a level and cool surface. This is obtained in the following manner:—Place a large shallow glass dish upon a tripod stand, and fill it to the brim with cold water; carefully cover the dish with a slab of plate glass, or a pane of window glass, and level it by placing the spirit-level in the centre and adjusting the screws of the tripod. Replace the level by a piece of filter paper, the size of the glass plates to be employed, and cover it with a shallow bell glass (Fig. 8).

Sterilisation of Glass Plates.—The glass plates are sterilised by filling the iron box (p. 27), and placing it in the hot-air steriliser, at 150° C., from one to two hours. As these plates are used

also for other purposes, a quantity ready sterilised should always be kept in the box.

Preparation of Damp Chambers.—The damp chambers for the reception of the inoculated plates are prepared thus:—Thoroughly cleanse and wash out with (1-1000) sublimate solution a shallow glass dish and bell (Fig. 7). Cut a piece of filter paper to line the bottom of the glass dish, and moisten it with the same solution.

In a glass beaker or an ordinary glass tumbler, with a pad of cotton wool at the bottom, place the tube containing the cultivation, the three tubes to be inoculated, and three glass rods which have been sterilised by heating in the flame of a Bunsen burner. Provide yourself with a strip of paper or a large label, a pencil, a pair of forceps, and inoculating needles. All is now ready at hand to commence the inoculation of the tubes.

Method of Inoculating the Test-tubes. Liquefy the gelatine in the three tubes by placing them in a beaker containing water at 30° C., or by gently warming them in the flame of the Bunsen burner. Keep the tubes, both before and after the inoculation, in the warm water, to maintain the gelatine in a state of liquefaction. Hold the tube containing the cultivation and a tube of the liquefied gelatine (to be called the "original") as nearly horizontal as possible between the thumb and index finger of the left hand. With the finger and thumb of the right hand loosen the plugs of

the tubes. Take the sterilised öse in the right hand and hold it like a pen. Remove the plug from the culture-tube by using the fourth and fifth fingers of the right hand as forceps, and place it between the fourth and fifth fingers of the left. Remove the plug of the "original" in the same way, placing it between the third and fourth fingers of the left hand (Fig. 28). With the öse take up a droplet

FIG. 28.—METHOD OF INOCULATING TEST-TUBES IN THE PREPARATION OF PLATE-CULTIVATIONS.

of the cultivation and inoculate the "original," twisting the öse several times in the liquid gelatine. Replace the plugs and set aside the cultivation. Hold the freshly inoculated tube between the fore-finger and thumb of either hand, almost horizontally, then raise it to the vertical, so that the liquid gelatine gently flows back. By repeating this motion and rolling the tube between the fingers and thumbs the micro-organisms which have been introduced are

distributed throughout the gelatine. Any violent shaking, and consequent formation of bubbles, must be carefully avoided. From the so-called "original" inoculate in the same manner a fresh tube of liquefied gelatine, introducing into it three droplets with a sterilised öse. This tube is then called the "first attenuation," or No. 1. After treating No. 1 as has been already described in the case of the original, the same process is repeated with a third tube, which is inoculated in the same way from No. 1. This is the "second attenuation" or No. 2, and in some cases a "third attenuation" is carried out from No. 2. The last tube must be inoculated in different ways, according to experience for different micro-organisms. Sometimes a sufficient separation of the micro-organisms is attained by inoculating the last tube with a fine straight needle dipped from one into the other from three to five times.

The next process consists in pouring out the gelatine on a glass plate and allowing it to solidify.

Preparation of the Gelatine-plates.—The directions to be observed in pouring out the gelatine are as follows:—

Place the box containing sterilised plates horizontally, and so that the cover projects beyond the edge of the table; remove the cover, and withdraw a plate with sterilised forceps; hold it between the finger and thumb by opposite margins, rapidly transfer it to the filter paper under the bell-glass, and

quickly replace the cover of the box. On removing the plug from "the original," an assistant raises the bell-glass, and the contents of the tube are poured on to the plate; with a glass rod the gelatine must be then rapidly spread out in an even layer within about half an inch of the margin of the plate. The assistant replaces the bell-glass, and the gelatine is left to set. Meanwhile a glass bench or metallic shelf is placed in the damp chamber, ready for the reception of the plate-cultivation, and when the gelatine is quite solid the plate is quickly transferred from under the bell-glass to the damp chamber; precisely the same process is repeated with tubes 1 and 2, and the damp chamber, labelled with the details of the experiment, is set aside for the colonies to develop. Not only plate-cultures should be carefully labelled with date and description, but the same remark applies equally to all preparations, tube-cultures, potato-cultures, drop-cultures, etc. In plate-cultivations write the source of the material, the date, and the number of inoculations; for example, thus:—

Finkler's comma-bacilli.

From tube-cultivation on "agar-agar," 5th February, 1885

Lower plate (Orig.) .	1 öse from cultivation.
Middle plate, No. 1 .	3 ösen from Orig.
Upper plate, No. 2 . .	3 ösen from No. 1.

Corresponding with the fractional cultivation of the micro-organisms obtained in this manner, the colonies will be found to develop in the course of a day or two, varying with the temperature of the room. The lower plate will contain a countless number of colonies which, if the micro-organism liquefies gelatine, speedily commingle, and produce, in a very short time, a complete liquefaction of the whole of the gelatine. On the middle plate, with the first attenuation, the colonies will also be very numerous, but retain their isolated position for a longer time; while on the uppermost plate, the second attenuation, the colonies are completely isolated from one another, with an appreciable surface of gelatine intervening [Plates VI. and VII.].

Examination of Plate-cultivations.—The macroscopical appearances of the colonies are best studied by placing the plate on the slab of blackened glass, or on the porcelain slab if the colonies are coloured.

To examine the microscopical appearances a selected plate is placed upon the stage of the microscope; it is better to have a larger stage than usual for this purpose. The smallest diaphragm is employed, and the appearances studied principally with a low power. These appearances should be carefully noted, and a rapid sketch of the colony made. The morphological characteristics of the micro-organisms of which the colony is formed can then be examined in the following way. A small

öse, or a platinum needle bent at the extremity into a miniature hook, is held like a pen, and the hand steadied by resting the little finger on the stage of the microscope. The extremity of the needle is steadily directed to the space between the lens and the gelatine without touching the latter, until,

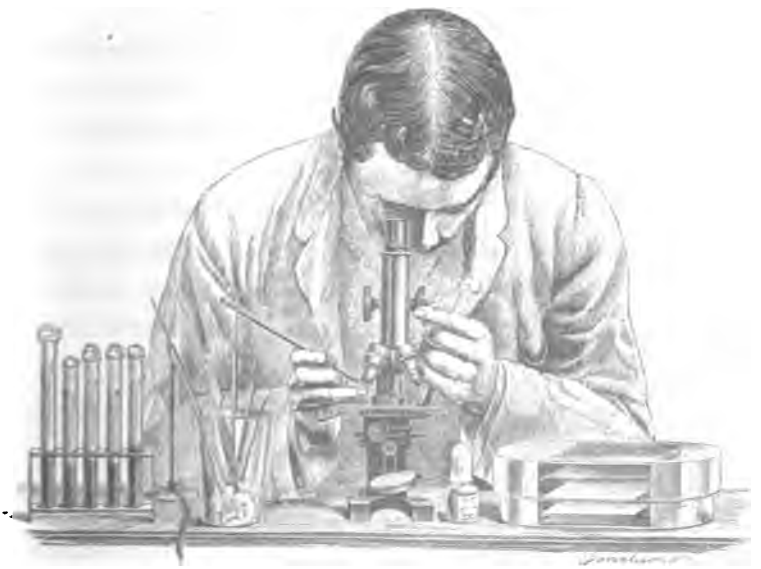


FIG. 29.—MICROSCOPICAL EXAMINATION OF COLONIES ON PLATE-CULTIVATIONS, AND METHOD OF ISOLATING COLONIES BY INOCULATION OF TEST-TUBES.

on looking through the microscope, it can be seen in the field, above or by the side of the colony under examination (Fig. 29). The needle is then dipped into the colony, steadily raised, and withdrawn. Without removing the eye from the microscope this manipulation can be seen to be successful by the colony being disorganised or completely re-

moved from the gelatine. It is, however, not easy to be successful at first, but with practice this can be accomplished with rapidity and precision. A cover-glass-preparation is then made in the manner already described, viz., by rubbing the extremity of the needle on a perfectly clean cover-glass and examining by Babes' rapid method, or by thinning out the micro-organisms in a droplet of sterilised water previously placed on the cover glass, drying, passing three times through the flame, and staining with a drop of fuchsine.

Inoculations should be made in test-tubes of nutrient gelatine and agar-agar, from the micro-organisms transferred to the cover-glass before it is dried and stained, from any remnants of the colony which was examined, or from other colonies bearing exactly similar appearances. In this way pure cultivations are established, and the macroscopical appearances of the growth in test-tubes can be studied. The plates should be replaced in the damp chambers as soon as possible; drying of the gelatine, or contamination with micro-organisms gravitating from the air during their exposure, may spoil them for subsequent examination. Nutrient agar-agar can also be employed for the preparation of plate-cultivations, but it is much more difficult to obtain satisfactory results. The test-tubes of nutrient agar-agar must be placed in a beaker with water and heated until the agar-agar is completely liquefied. The gas is then

turned down and the temperature of the water allowed to fall until the thermometer stands just above 50° C. The water must be maintained at this temperature, and the test-tubes must be in turn rapidly inoculated and poured out upon the glass plates, as already described. Glass plates may also be employed in a much simpler method. The nutrient jelly is liquefied, poured out, and allowed to set. A needle charged with the material to be inoculated is then streaked in lines over the surface of the jelly. This method is of especial value in inoculating different organisms side by side, and watching the effect of one upon the other, or a micro-organism in this way may be sown upon gelatine which has been already altered by the growth of another micro-organism; the change produced in the gelatine, as in the case of the fluorescing bacillus, extending far beyond the limits of the growth itself (Plate VIII., Fig. 3).

Nutrient jelly may also be spread out on sterilised microscopic slides and inoculated as just described, or cultivations may be made in shallow glass dishes, glass capsules, etc., which must be sterilised on the principles already laid down, and after inoculation placed in damp-chambers for the growths to develop.

(c) PREPARATION AND EMPLOYMENT OF STERILISED POTATOES, POTATO-PASTE, BREAD-PASTE, VEGETABLES, FRUIT, AND WHITE OF EGG.

Potato Cultivations.—Sterilised potatoes form an excellent medium for the cultivation of many micro-organisms, more especially the chromogenous species. Potato-cultivations also give in some cases very characteristic appearances, which are of value in distinguishing bacteria which possess morphological resemblances [Plates IX. and X.]

Preparation of Sterilised Potatoes.—Potatoes, preferably smooth-skinned, which are free from "eyes" and rotten spots, should be selected. If they cannot be obtained without eyes and spots, these must be carefully picked out with the point of a knife with as little destruction of the surface as possible. The potatoes are well scrubbed with a stiff brush and allowed to soak in sublimate solution for half an hour. They are then transferred to the potato-receiver and steamed in the steam-steriliser for twenty minutes to half an hour, varying according to the size of the potatoes. When cooked, the potato-receiver is withdrawn and left to cool, the potatoes being retained in it until required for use.

Damp chambers are prepared ready for their reception, the vessels being cleansed and washed with sublimate as described for plate-cultivations. Small glass dishes of the same pattern as the

large ones (Fig. 13) may be employed for single halves of potatoes. Potato knives and several scalpels which have been sterilised in an Israel's case by heating them in the hot-air steriliser to 150° for one hour, must be ready to hand. The potato knives may also be sterilised by heating them in the flame of a Bunsen burner and placing them on their backs with their blades projecting over the edge of the table. Scalpels may be sterilised in the same way and laid upon a sterilised glass plate and covered with a bell-glass. It must not be forgotten, however, that heating the blades in the flame destroys the temper of the steel, and therefore knives and other instruments should preferably be sterilised in the hot-air steriliser, enclosed in an Israel's case, or simply enveloped in cotton wool.

Inoculation of Potatoes.—The coat sleeves should be turned back, and the hands, after a thorough washing with good lathering soap, be dipped in sublimate solution. An assistant opens the potato receiver, and a potato is selected, and held between the thumb and index finger of the left hand (Fig. 30). With the knife held in the right hand, the potato is almost completely divided in the direction which will give the largest surface. The assistant raises the cover of the damp chamber, and the potato is introduced, and while withdrawing the knife, allowed to fall apart. The cover is quickly replaced, and another

potato treated in the same way is placed in the same damp chamber. The four halves are then quite ready for inoculation. As an extra precaution the left hand is again dipped in sublimate, and one half of a potato is taken up between the tips of the thumb and index finger, care being taken to avoid touching the cut surface. Holding

FIG. 30.—METHOD OF DIVIDING POTATOES.

it with its cut surface vertical, a small portion of the substance to be inoculated is placed on the centre with a sterilised needle or öse. With a sterilised scalpel the inoculated substance is rapidly spread over the surface of the potato with the flat of the blade to within a quarter of an inch of the margin, and the potato is then as

quickly as possible replaced in the moist chamber. With another sterilised scalpel a small portion of the potato from the inoculated surface of the first half is in the same way spread over the surface of the second half, this forming as in plate-cultivations a "first attenuation." Exactly the same is repeated with a third potato, and even a fourth, so that a still further attenuation or fractional cultivation of the micro-organisms may be obtained. In some cases it is necessary to place the cultures in the incubator, others grow very well at the temperature of the room. As in plate-cultivations, the potato may also be inoculated by simply streaking it in lines with a needle charged with the material to be inoculated.

POTATO-PASTE.

Potato-paste is sometimes employed where it is desirable to obtain an extensive growth of certain bacteria. The potatoes are boiled for an hour, and the floury centre squeezed out of their skins. This is then mashed up with sufficient sterilised water to produce a thick paste, and is heated in the steam steriliser for half an hour for three successive days.

BREAD-PASTE.

Some micro-organisms, more especially mould fungi, grow very well on bread-paste. This is prepared by removing the crust from a stale loaf

of bread and allowing it to dry in the oven. It is then broken up, and reduced to a fine powder with a pestle and mortar. Small, carefully cleansed, conical, or globe-shaped flasks are plugged with cotton wool and sterilised in the oven. When cool a small quantity of the powder is placed in them, and sterilised water added in the proportion of one part for every four of the powder. The paste is sterilised by steaming in the steriliser at 100° C. for half an hour for three successive days. The flasks can be reversed, and are inoculated in the usual way with a platinum needle.

CULTIVATIONS ON VEGETABLES, FRUIT, WHITE OF EGG, ETC.

Boiled carrots and other vegetables, and various kinds of stewed fruit, are also occasionally employed for the cultivation of bacteria. The sterilisation of these media must be carried out on the principles already explained. White of egg may be poured out on sterilised glass plates, or in shallow glass dishes, boiled in the steam-steriliser and after inoculation, placed in a damp chamber.

(D) PREPARATION AND EMPLOYMENT OF STERILE BLOOD SERUM.

The tubercle-bacillus, the bacillus of glanders, and a few other micro-organisms, thrive best when culti-

vated on solid blood serum. This medium has the additional advantage of remaining solid at all temperatures. The technique required for its preparation and sterilisation is as follows:—Several cylindrical vessels, about 20 cm. high, are thoroughly washed with sublimate solution (1-1000), and then with alcohol, and finally rinsed out with ether. The ether is allowed to evaporate, and the vessels are then ready for use. The skin of the animal selected—calf, sheep, or horse—is washed with sublimate at the seat of operation, and the bleeding is performed with a sterilised knife. The first jet of blood from the vein is rejected, and that which follows is allowed to flow into the vessels until they are almost full. The ground-glass stoppers, greased with vaseline, are replaced, and the vessels set aside in ice, as quickly as possible, for from twenty-four to thirty hours. By that time the separation of the clot is completed, and the clear serum can then be transferred to plugged sterile test-tubes. These should be filled with a sterilised pipette for about a third of their length, and are then placed in Koch's slow steriliser with the temperature maintained for an hour at 58° C. The same process is repeated for six successive days, the temperature on the last day being gradually raised to 60°. This completes the sterilisation, but to solidify the serum it is necessary to arrange the tubes in the inspissator at the angle required. The temperature of this apparatus is kept between 65°

and 68° C. Directly solidification takes place the tubes must be removed, and they should then present the character of being hard, solid, of a pale straw colour, and transparent. A little liquid collects at the lowest point, and the serum is sometimes milky in appearance at its thickest part. The serum may not only be employed in test-tubes, but also in small flasks, glass capsules, or other vessels, all of which must be cleansed and sterilised in the usual way. Hydrocele fluid and other serous effusions may be prepared in the same manner, or gelatine may be added to the serum in the proportion of 5 per cent.

Inoculation of the Tubes.—A small portion of the material to be inoculated is taken up with a sterilised needle or öse, and drawn in lines over the sloping surface of the serum; or a minute piece of tissue, tubercle, etc., may be introduced into the tube and deposited on the surface of the nutrient medium. The precautions that are to be observed in isolating the material to be inoculated will be referred to later (p. 112).

Liquid Media.

(E) Preparation of Sterilised Bouillon, Liquid Blood Serum, Urine, Milk, Vegetable Infusions, and Artificial Nourishing Liquids.

Nutrient liquids are still largely employed, and by some observers even in preference to the solid

media advocated by Koch. It must not be supposed, however, that the methods of cultivation in liquids are discarded entirely by the German school, for there is no more instructive method than the employment of so-called drop-cultures. For inoculation experiments where the presence of gelatine is undesirable, for studying the physiology and chemistry of bacteria and where for any object a rapid growth of micro-organisms is necessary, the employment of liquid media is not only advisable, but is absolutely necessary. Liquid media comprise two distinct groups—*natural* and *artificial*. The natural group includes meat broths, blood, urine, milk, and vegetable infusions; the artificial are solutions built up from a chemical formula representing the essential food constituents.

Natural Media.

Bouillon.—A broth or bouillon of beef, pork, or chicken may be made in the same manner as described for the preparation of gelatine-peptone-broth, with simply omission of the gelatine. After the neutralisation with carbonate of soda solution drop by drop, the flask of broth is placed in the steam steriliser for half an hour at 100° C. A clear liquid results on filtration, which is transferred to plugged sterilised flasks or test-tubes, and sterilisation effected by exposing them in the steam steriliser

for half an hour at 100° C. for two or three successive days.

Liquid Blood Serum.—The preparation of sterile blood serum has already been described. It may be used for cultivation, especially in the form of drop-cultivations, before the final treatment by which it is solidified. Hydrocele fluid, peritonitic and pleuritic effusions, can also be employed after sterilisation in the steam steriliser. The fluid should be withdrawn with a sterilised trocar and canula, and received into plugged sterilised flasks.

Urine.—In order to obtain urine free from micro-organisms the following precautions must be observed:—The orifice of the urethra must be thoroughly cleansed with sublimate solution. The first jet of urine should be rejected, and the rest received into sterilised vessels, which must be quickly closed with sterile plugs. If these precautions be not attended to the urine must be rendered sterile by the means described for the sterilisation of bouillon.

Milk.—If milk has been drawn into sterile flasks after thoroughly cleansing and disinfecting the teats and hands, it may be kept without change. If procured without these precautions it must be steamed in the steriliser for half an hour for five successive days.

Vegetable Infusions. — Infusions of hay, cucumber, and turnip are used for special purposes, and more rarely decoctions of plums, raisins, malt, and horse-dung. They are mostly prepared

by boiling with distilled water, after maceration for several hours. The filtrate is received into sterile flasks and sterilised in the usual way in the steam steriliser.

Artificial Fluids.

Pasteur's Fluid.—This solution is prepared by mixing the ingredients in the following proportions:—

Distilled water	100
Pure cane sugar	10
Ammonium tartrate	1
Ash of yeast	·075

Cohn-Mayer Fluid.—Mayer's modification of the nourishing fluid employed by Cohn is as follows:—

Distilled water	20
Phosphate of potassium	·1
Sulphate of magnesium	·1
Tribasic calcium phosphate	·01
Ammonium tartrate	·2

(F) Methods of storing and employing liquid media; Lister's flasks, Aitken's test-tubes, Sternberg's bulbs, Pasteur's apparatus, Miquel's bulbs; drop-cultures.

Cultivations in liquid media can be carried on in test-tubes, but it is more satisfactory to employ

special forms of flasks, bulbs, U tubes, etc. As test-tubes and flasks containing liquid media cannot be inverted, inoculation with a sterilised needle must be carried out as rapidly as possible, with the additional precaution of closed windows and doors.

Lister's Flasks.—These flasks (p. 31) were especially introduced by Sir Joseph Lister as a means of storing liquid nutrient media. They are so constructed that after removal of a portion of the contents, or restoring the vessel to the vertical position, a drop of liquid always remains in the extremity of the nozzle, which prevents the regurgitation of unfiltered air.

Sternberg's Bulbs.—The method of introducing liquid into the bulbs employed by Professor Sternberg, and of sterilising and inoculating it, is as follows:—The bulb is heated slightly over the flame, and the extremity of the neck, after breaking off the sealed point, is plunged beneath the surface of the liquid. As the air cools the liquid is drawn into the bulb, usually filling it to about one-third of its capacity. The neck of the flask is again sealed up, and the liquid which has been introduced is sterilised by repeatedly boiling the flasks in the water-bath. They should then be placed in the incubator for two or three days, and if the contents remain transparent and free from film, they may be set aside as stock-bulbs, to be used when required.

To inoculate the liquid in the bulb the end of the neck is heated to sterilise the exterior, the bulb

is gently warmed, and the extremity of the neck nipped off with a pair of sterilised forceps. The open extremity is plunged into the liquid containing the micro-organism, a minute quantity enters the tube and mingles with the fluid in the bulb, without fear of contamination by atmospheric germs. The extremity of the neck is once more sealed up in the flame of a Bunsen burner.

Aitken's Tubes.—These tubes are plugged and sterilised, and the nutrient medium introduced as into ordinary test-tubes. Instead of withdrawing the cotton wool plug they are inoculated by means of the lateral arm. The sealed extremity of the arm is nipped off with sterilised forceps, and the inoculating needle is carefully introduced through the opening thus made. It is directed along the arm until it touches the opposite side of the test-tube, where it deposits the material with which it was charged. The needle is withdrawn, and the end of the lateral arm again sealed up in the flame; the test-tube is then tilted until the liquid touches the deposited material; on restoring the tube to the vertical the material is washed down into the body of the nutrient liquid.

Pasteur's Apparatus. — Special forms of tubes, bulbs, and pipettes are employed by the school of Pasteur. The tubes are provided with lateral or with curved arms drawn out to a fine point, and with slender necks plugged with cotton wool. A double form shaped like a tuning fork,

each limb with a bent arm, is a convenient form for storing sterilised bouillon. The sealed end of an arm is nipped off with sterilised forceps, the sterile bouillon aspirated into each limb, and the arm again sealed in the flame; a series of such tubes can be arranged upon a rack on the working table.*

Bulbs with a vertical neck drawn out to a fine point; others with a neck bent at an obtuse angle plugged with cotton wool, and a lateral curved arm drawn out to a fine point, are also employed. For a description of these various vessels and their special advantages the works of Pasteur and Duclaux must be consulted.

Miquel's Bulbs.—The *tube à boule* of Miquel† is also a very useful form. It consists of a bulb of 50 cc. capacity blown in the middle of a glass tube. The part of the tube above the bulb is contracted about half way between the bulb and its extremity, and can either be left quite straight or can be made to curve slightly. On either side of the contraction the tube is plugged with asbestos. The portion of the tube below the bulb is S shaped, and drawn out at its extremity into a fine point. The bulbs are charged with nutrient liquid and inoculated by aspiration, and the point of the S tube sealed in the flame of a Bunsen burner.

Drop-Cultures.—This method of cultivation

* Duclaux, *Ferments et Maladies.* 1882.

† Miquel, *Les Organismes Vivants de l'Atmosphère.* 1883.

has already been referred to as a particularly instructive one. It enables us to study many of the changes which take place during the life history of micro-organisms. This is illustrated, for example, by the anthrax bacillus, where we can watch the gradual growth of a single bacillus into a long filament, and the subsequent development of bright oval spores. It is necessary carefully to observe the minutest details to maintain the cultivation pure. An excavated slide is thoroughly cleaned, and then sterilised by being held with the cupped side downwards in the flame of the Bunsen burner. A ring of vaseline is painted round the excavation, and the slide is then placed under a glass bell. Meanwhile a carefully cleansed cover-glass is also sterilised by passing it through the flame, and should be deposited on the plate of blackened glass. With a sterilised öse a drop of sterile bouillon is transferred to the cover glass, and this is inoculated by touching it with a sterilised needle charged with the material without disturbing the form of the drop. It is quite sufficient just to touch the drop instead of transferring a visible quantity of blood, juice, or growth, as the case may be. The slide is then inverted and placed over the cover-glass, so that the drop will come exactly in the centre of the excavation, and is gently pressed down. On turning the slide over again the cover-glass adheres, and an additional layer of vaseline is painted round the edges of the cover-glass itself. The slide must be

labelled, and, if necessary, placed in the incubator, and the results watched from time to time. Instead of bouillon liquid blood serum may also be employed in this form of cultivation. If it be required to preserve the drop cultivation as a microscopic preparation, the cover-glass is gently lifted off and allowed to dry. Any vaseline adhering to the cover-glass should be wiped off, and the cover-glass can then be passed through the flame and stained in the usual manner.

Moist-Chambers.—Unless drop-cultures are very carefully prepared, they are liable to dry up, if kept for examination for several days. Many therefore prefer employing a moist-chamber. There are several different forms in use.

The drop-culture slide may be converted into a moist-chamber by having a deep groove cut round the circumference of the concavity. This groove is filled with sterilised water by means of a pipette. A ring of vaseline is painted with the camel's-hair brush outside the groove, and the cover-glass with the drop-cultivation is inverted and placed over the concavity. This form is very useful, as the slide can be easily cleansed and effectually sterilised by holding it in the flame of the Bunsen burner.

A very simple form of moist-chamber which may be used in some cases, but possesses the disadvantage of not admitting of sterilisation by heat, may be constructed as follows* :—

* Schäfer's *Course of Practical Histology*. 1877.

A small piece of putty or modelling wax is rolled into a cord about two inches long and $\frac{1}{8}$ inch thick. By uniting the ends a ring is formed, which is placed on the middle of a clean glass slide (Fig. 31). A drop of water is placed in the centre of the ring, and the cell roofed in by applying the cover-glass.

A somewhat similar cell in form, which has the advantage of permitting of thorough cleansing, may

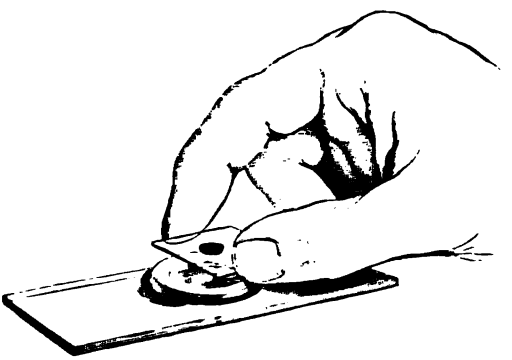


FIG. 31.—METHOD OF FORMING A SIMPLE MOIST-CHAMBER.

be constructed by cementing a glass ring with flat surfaces to an ordinary slide. Vaseline is applied with a camel's-hair brush to the upper surface of the ring, and one or two drops of water placed with a pipette at the bottom of the cell. The cover-glass, with the preparation, is then inverted over the cell and gently pressed down upon the glass ring. The vaseline renders the cell air-tight, and, to a certain extent, fixes the cover-glass to the ring.

Warm Stages.—To apply warmth while a preparation is under continuous observation we must

either place the microscope bodily within an incubator, with the eyepiece protruding through an opening, so that we may observe what is going on without moving the preparation, or we must employ some means of applying heat directly to the preparation.

A simple warm stage may be made of an oblong copper plate, two inches long by one inch wide, from one side of which a rod of the same material projects. The plate has a round aperture in the middle, half an inch in diameter, and is fastened to

FIG. 32.—SIMPLE WARM STAGE.

an ordinary slide with sealing wax. The drop to be examined is placed on a large-sized cover-glass and covered with a smaller one. Olive oil or vaseline is painted round the edge of the smaller cover-glass to prevent evaporation, and the preparation is placed over the hole in the plate (Fig. 32).

The slide bearing the copper plate is clamped to the stage of the microscope (Fig. 33). The flame of a spirit lamp is applied to the extremity of the rod, and the heat is conducted to the plate and thence transmitted to the specimen. That the

temperature of the copper plate may be approximately that of the body, the lamp is so adjusted that a fragment of cacao butter and wax placed close to the preparation is melted.

For more accurate observations, the apparatus shown in Fig. 34 may be employed. The vessel *f*,

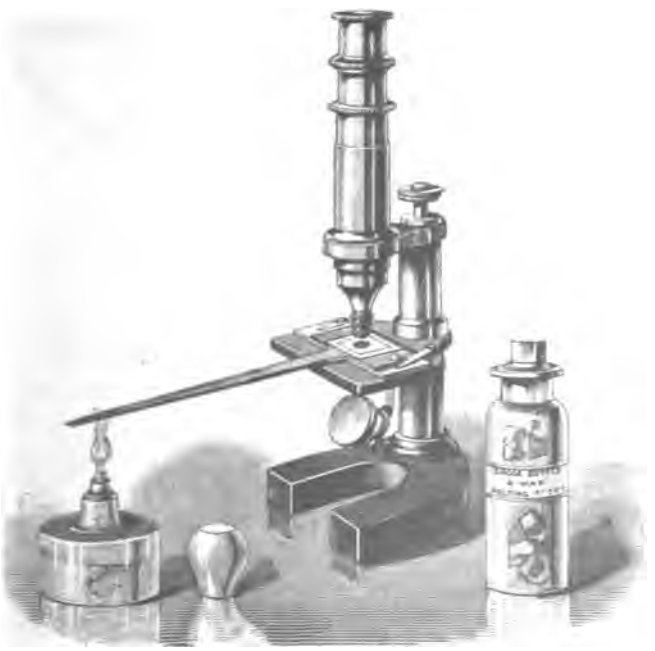


FIG. 33.—SIMPLE WARM STAGE SHOWN IN OPERATION.

filled with water which has been boiled to expel the air, is heated by means of a gas-flame at *g*. The warmed water ascends the indiarubber tube *c* to the brass box *a*. The box is pierced by a tubular aperture to admit light to the object, and has an exit tube *c'*, by which the cooled water from the

stage returns to be reheated by the flame *g*. At *d* is a gas regulator, so that a constant temperature at any desired point can be maintained.

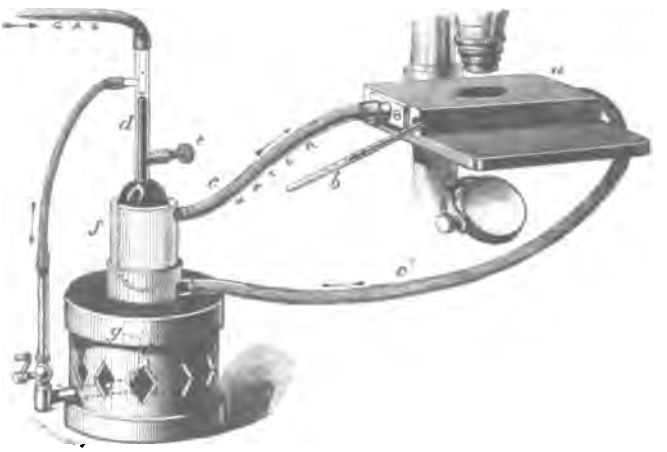


FIG. 34.—SCHÄFER'S WARM STAGE.

Another form in which warm water or steam can be used for heating, and by the employment of

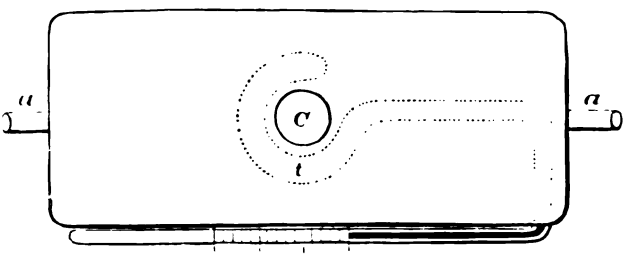


FIG. 35.—STRICKER'S WARM STAGE.

iced water also used for observing the effects of cold, is shown in Fig. 35. It consists of a hollow rectangular box, with a central opening (*C*) permitting

the passage of light. The water makes its exit and entrance at the side tubes *a*, *a*, and the temperature is indicated by a thermometer in front.

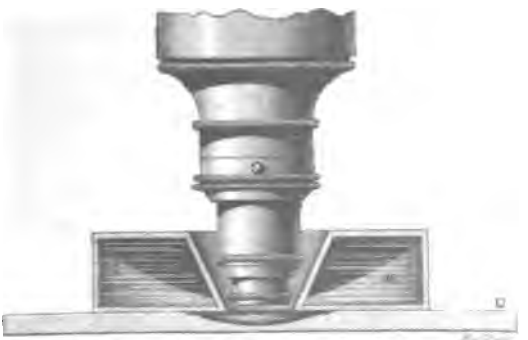


FIG. 36.—SECTION OF ISRAEL'S WARMING APPARATUS AND DROP-CULTURE SLIDE.

Israel's Warming Apparatus.—It is obvious that in employing very high powers a difficulty will

FIG. 37.—ISRAEL'S WARMING APPARATUS.

be presented by the warm stages just described owing to their interference with the illumination.

To overcome this an apparatus has been constructed by which the slide is warmed from above.*

The drop-culture slides are provided with a shallow groove ·1 mm. deep and 1 mm. broad, cut round the concavity. Into this the cover-glass fits,

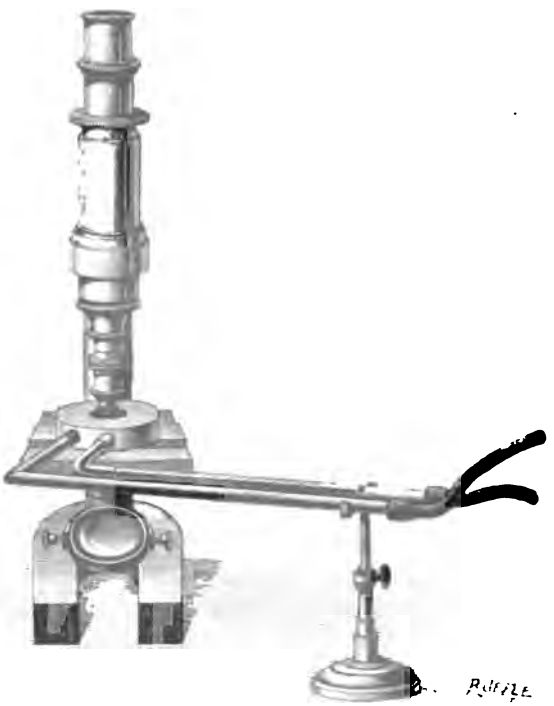


FIG. 38.—ISRAEL'S WARMING APPARATUS IN OPERATION.

so that its upper surface is flush with that of the slide. The heating apparatus consists of a flat disk-shaped box with a central conical aperture (Fig. 36).

The entrance and exit pipes are fixed on at a

* Israel, *Zeitsch. f. Wiss. Mikrosc*, ii., pp. 459—63. 1885.

right angle to the side (Fig. 37). The former, *z*, is of metal, and the latter, *a*, of glass fitted with a thermometer, the bulb of which, *k*, is contained within the box. A partition, *s*, keeps up a current between the openings of the pipes, which are supported on a stand and connected by tubing with the hot water supply (Fig. 38).

A mixture of paraffine and vaseline is recommended for indicating the temperature of the chamber, and experience has shown that if a tem-

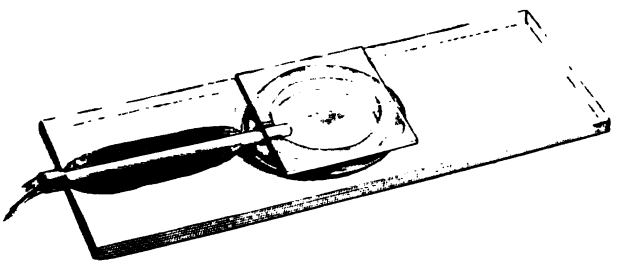


FIG. 39.—SIMPLE GAS CHAMBER.

perature of 37° C. be required the temperature of the water in the box must range between 42° and 47° C.

Gas Chambers.—To investigate the action of gases or vapours upon micro-organisms, a modification of the simple moist chamber (Fig. 31), may be employed (Fig. 39).

A piece of glass tubing is first fixed to the slide by means of sealing wax, and the ring of putty is so placed as to include the end of this, leaving a small interval at the side, or a little notch is made in the putty opposite, so as to afford an exit for the gas or vapour (Fig. 40).

A more complicated apparatus, combining both a warm stage and a gas chamber, is shown at Fig. 41. This consists of a rectangular piece of ebonite EE fixed to a brass plate which rests on the stage of the microscope. On the upper surface of the ebonite is another brass plate *p*, with an aperture *c* leading into a brass tube closed below by a piece of glass. To heat the apparatus the copper wire B is placed

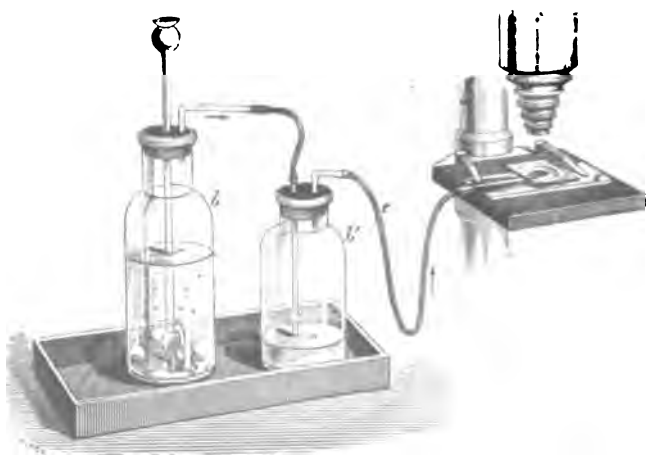


FIG. 40.—GAS CHAMBER IN USE WITH APPARATUS FOR GENERATING CARBONIC ACID.

on the tube *a*, and its extremity heated by the flame of the lamp. The nearer the lamp to the stage the higher the temperature, which is indicated by the thermometer *t*. To employ it as a gas chamber the wire B is laid aside and the gas is conducted into the chamber by the tube *a'* and escapes by the tube *a*.

Application of Electricity.—To study the

effect of electricity we may prepare a drop-culture in the moist-chamber (Fig. 42). The cover-glass to be used is provided with two strips of tinfoil,

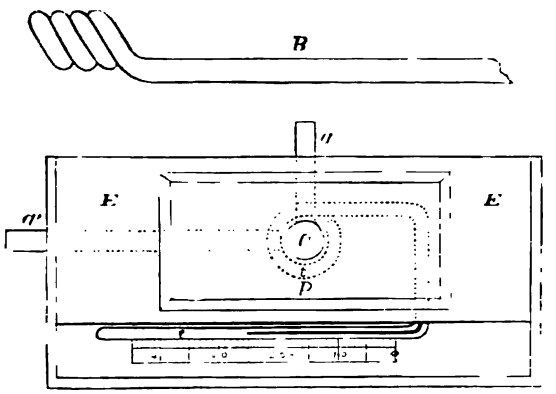


FIG. 41.—STRICKER'S COMBINED GAS CHAMBER AND WARM STAGE.

which are isolated from the brass of the microscope, and so arranged that a current of electricity may be passed through them (Fig. 43).

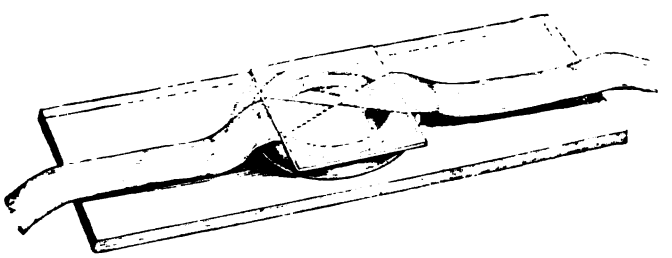


FIG. 42.—SIMPLE MOIST-CHAMBER ADAPTED FOR TRANSMISSION OF ELECTRICITY.

A much simpler plan, which may also be employed, is to take an ordinary glass slide and coat the surface with gold size. The slide is then pressed

firmly down on gold leaf or tin-foil and allowed to dry. When dry, the metal is scraped away, leaving

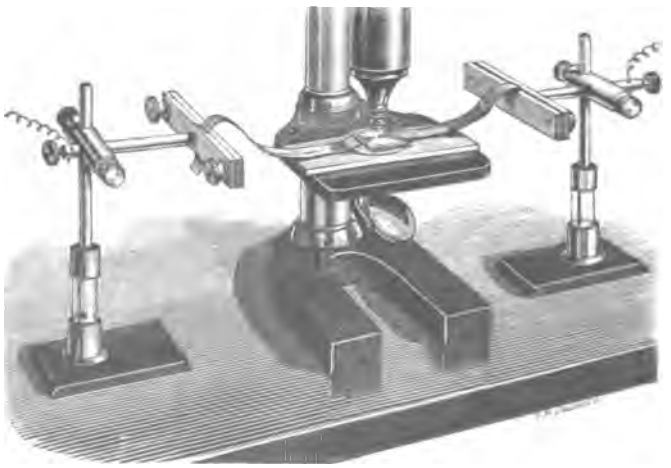


FIG. 43.—APPARATUS ARRANGED FOR TRANSMITTING ELECTRICITY.

two triangles with a small interval between them, as in Fig. 44.

FIG. 44.—SLIDE WITH GOLD-LEAF ELECTRODES.

The liquid containing the micro-organisms is placed between the electrodes, covered with a cover-glass, and then subjected to the electric current.

CHAPTER VI.

EXPERIMENTS UPON THE LIVING ANIMAL.

To carry out the last of Koch's postulates, and so complete the chain of evidence in favour of the causal relation of micro-organisms to disease, and to study the mode of action of a pathogenic bacterium, it is necessary to introduce into a living animal a pure cultivation of the micro-organism in question. For this purpose various animals are employed—such as mice, guinea-pigs, rabbits, pigeons, and fowls.

Inhalation of Micro-organisms. — The animals may be made to inhale an atmosphere impregnated with micro-organisms by means of a spray. In this way Friedländer succeeded in administering the bacteria of pneumonia to mice, and the production of tuberculosis by experimental inhalation has thrown light upon the clinical records of cases reported as instances of the infectiousness of phthisis.

Administration with Food.—A sheep fed upon potatoes which have been the medium for the cultivation of the anthrax bacillus dies in a

few days. Similarly, animals fed upon the nodules of bovine tuberculosis become tubercular, and even the flesh and milk of tuberculous animals will occasionally set up tuberculosis.

Cutaneous and Subcutaneous Inoculation.—Cutaneous inoculation may be carried out by making a superficial wound, and inoculating it with a sterilised platinum needle, charged with the micro-organisms to be inoculated. Another simple method is to take a sterilised knife, infect the point with the material to be inoculated, and then make a minute wound or incision. In either case a situation should be selected, such as the root of the ear, which cannot be licked by the animal after the operation.

Subcutaneous inoculation is very simple and effectual, and consequently the method most frequently employed. The animal selected—for example, a guinea-pig—is held by an assistant, who covers it with a towel, leaving only the hinder extremities exposed. By so doing, and gently laying it upon its back, with its head low, a guinea-pig passes apparently into a state of hypnotism, and the trivial operation can be performed with little or no movement on the part of the animal. From a spot on the inner side of the thigh the hair is cut close with a small pair of scissors curved on the flat, and the skin must be thoroughly purified with sublimate solution. A small fold of skin is then pinched up

with a pair of sterilised forceps, and with a pair of sharp sterilised scissors, or with a tenotomy knife, a minute incision is made. A sterilised platinum öse is charged with the material to be inoculated, and the loop is gently inserted under the skin, forming a small pocket in the subcutaneous tissue. The needle is then withdrawn, and the sides of the wound gently pressed into apposition. In a mouse the same process is adopted, with the exception that the root of the tail is the usual site of the operation. In a method suggested by Koch an assistant can be dispensed with: a glass bell reversed is placed as a cover to a wide-mouthed glass jar, in which a mouse is held by the tail with a pair of forceps, while the cover is so placed over the mouth of the jar as to leave a small interval near the rim uncovered. The mouse rests with its head downwards and with its feet against the inner wall of the jar, and in the interval between the cover and the rim the root of the tail is exposed, and must be cleansed and treated as already described.

Special Operations. — In many cases it is absolutely necessary to perform an operation of greater severity. After the administration of an anæsthetic, infective material may be introduced into the peritoneal cavity by the performance of abdominal section, or injected into the duodenum in the manner employed in the case of Koch's

comma bacilli by Nicati and Rietsch. In such cases antiseptic precautions must be rigidly followed, and use made of iodoform and other antiseptic dressings. The disinfection of the skin of the animal, of the instruments employed, and of the hands of the operator, are details essential to secure success. To inoculate tubercular matter, sputum may be rubbed up with distilled water, filtered, and the filtrate injected into a tracheal fistula, or the first steps of the operation of iridectomy may be performed, and tubercular material inserted in the anterior chamber of the eye. The advantage of the latter method consists in that it enables the results and changes to be observed from day to day. A cultivation of micro-organisms may also be mixed with sterilised water, and then injected with a syringe directly into the circulation. In rabbits this may be performed without difficulty by injecting the large vein at the base of the ear with a Pravaz' syringe. Before every inoculation the instruments must be sterilised, as already explained, by employing an Israel's case, and after each operation all instruments should be placed in sublimate solution, wiped dry, and sterilised in the hot-air steriliser, before they are put away. If these precautions are not observed, instances of accidental infection are sure to occur.

CHAPTER VII.

EXAMINATION OF ANIMALS EXPERIMENTED UPON AND THE METHODS OF ISOLATING MICRO-ORGANISMS FROM THE LIVING AND DEAD SUBJECT.

METHOD OF DISSECTION AND EXAMINATION.

ALL animals that die after an experimental inoculation should be examined immediately after death. Every precaution must be taken, in conducting the dissection, to exclude extraneous micro-organisms, and all instruments employed must have been sterilised in the hot-air steriliser, or heated in the Bunsen burner. If a mouse, for example, has died after an inoculation, it should be at once pinned out by its feet on a slab of wood or in a gutta-percha tray, and bathed with sublimate solution. In the same way, before examining a dead rabbit, a stream of sublimate should be directed over it to lay the fur, which otherwise interferes with the dissection. The hair should be cut away with sterilised scissors from the seat of inoculation, which is the first part to be examined, and any suppuration, hæmorrhage, œdema, or other pathological

change should carefully be noted. From any pus or exudation that may be present, material for inoculations should at once be taken, and cover-glass-preparations made for microscopical examination.

To examine the internal organs and to make inoculations from the blood of the heart or spleen, the skin is cut through from below upwards in the median line of the abdominal and thoracic regions. The abdominal cavity is then opened, and the walls pinned back on either side of the animal. Any abnormal appearances should be noted, and especially the state of the spleen should be examined, by turning the intestines aside. After noting its appearances it should be removed with sterilised forceps and scissors, and deposited upon a sterilised glass slide. After washing it with sublimate solution by means of a camel's hair brush or strip of filter paper, it should be incised with sterilised scissors; the pulp may be squeezed out from the cut surface, and test-tubes of nutrient gelatine and agar-agar can be inoculated from it, and, if necessary, potato and drop-cultivations also established. Precisely the same care must be taken in examining lymphatic glands, tubercles, or pathological nodules; any chance putrefactive micro-organisms on the surface are destroyed by the sublimate solution, and a section is then made, and a minute fragment snipped out of the centre of the nodule, to be examined or transferred to the nutrient medium. The examination of the thorax is made by cutting

through the ribs on either side of the sternum with sterilised scissors, and turning the sternum up where it will be out of the way. The pericardium is then opened, and the right auricle or ventricle pierced with the point of a sterilised scalpel, and inoculations and cover-glass-preparations are made from the blood which escapes.

The lungs also require to be especially studied. They should be incised with a sterilised scalpel, and inoculations and cover-glass-preparations made from the cut surface. It may be necessary to embed a piece of lung or fragment of spleen, so that it shall be free from air. This may be done by isolating a fragment with the precautions just described and depositing it upon the surface of a test-tube of nutrient agar-agar. The contents of another tube, which have been liquefied, and allowed to cool almost to the point of gelatinisation, must then be poured over it. From a potato a little cube must be cut, the tissue deposited in the trough thus formed, and the cube replaced. Blood may also be taken directly from a vein by laying it bare by dissection, making a small section with sterilised scissors, and inserting an öse, the needle of a Pravaz' syringe, a capillary tube, or the extremity of the capillary neck of a Sternberg's bulb. If the cultivation is contaminated by the presence of putrefactive or other micro-organisms they must be isolated subsequently by carrying out a series of plate-cultivations.

Having completed the dissection, the organs of such a small animal as a mouse may be removed *en masse* and transferred to absolute alcohol for subsequent examination. In other cases it may be only necessary to reserve portions of each organ. In any case it should be remembered that with a virulent micro-organism, *e.g.*, anthrax, any remaining part of the animal should be cremated, and the hands and all instruments should be thoroughly disinfected.

Isolation of Micro-organisms from the Living Subject.—Micro-organisms in the living subject may be isolated from pus of abscesses, or other discharges, and from the blood and tissues. Abscesses should be opened, and other operations performed, when practicable, with Listerian precautions, and a drop of the discharge taken up with an öse or capillary pipette as already explained.

To make a cultivation from the blood of a living person, the tip of a finger must be well washed with soap and water and bathed with strong sublimate, or 1 in 20 carbolic, solution. Venous congestion is produced by applying an elastic band or ligature to the finger, which is pricked with a sterilised sewing needle. From the drop of blood which exudes the necessary inoculations and examinations can be made.

PART II.

GENERAL BIOLOGY OF BACTERIA.

CHAPTER VIII.

GENERAL MORPHOLOGY AND PHYSIOLOGY.

BACTERIA may be considered as minute vegetable cells destitute of nuclei. They are distinguished from animal cells by being able to derive their nitrogen from ammonia compounds, and they differ from the higher vegetable cells in being unable to split up carbonic acid into its elements, owing to the absence of chlorophyll. Von Engelmann and Van Tieghem include among the bacteria certain organisms, named by them *Bacterium chlorinum*, *Bacterium viride*, and *Bacillus virens*, which are coloured green by this substance; but further researches are required before any conclusions are definitely arrived at as to the place of these particular organisms in the vegetable kingdom. It is quite possible that they may be Algæ, and they will, therefore, find no place in the classification which will be here adopted.

Chemical composition.—For our knowledge of the composition of bacteria we are chiefly indebted to Nencki. Their constituents are found on

analysis to vary slightly, according to whether the bacteria are in zooglœa or in the active state. In the latter condition they are said to consist of 83·42 per cent. of water. In one hundred parts of the dried constituents there are the following:—

A nitrogenous body . . .	84·20
Fat	6·04
Ash	4·72
Undetermined substances . .	5·04

This nitrogenous body is called *Mycoprotein*, and consists of

Carbon	52·32
Hydrogen	7·55
Nitrogen	14·75

but no sulphur or phosphorus.

The nitrogenous body appears to vary in different species, for in *Bacillus anthracis* a substance has been obtained which does not give the reactions of mycoprotein, and, therefore, is distinguished as anthraxprotein.

Considering bacteria as cells, we may speak of the cell-wall and the cell-contents.

Cell-wall.—The cell-wall consists of cellulose, or according to Nencki in the putrefactive bacteria of mycoprotein. It may be demonstrated by the action of iodine, which contracts the protoplasmic contents, and renders the cell-wall visible. The author has taken advantage of the action of iodine to differentiate by staining the sheath of the *Bacillus*

anthracis from its contents. If we stain cover-glass preparations of this bacillus by the method of Gram, we get the following results. By the first solution the rods are uniformly stained blue; by subjecting them to the iodine solution, the protoplasmic contents are contracted, while the next solution, alcohol, decolorises the sheath, which may be then stained in contrast with eosin.

FIG. 45.

FROM A PREPARATION OF BACILLUS ANTHRACIS, DOUBLE STAINED WITH GENTIAN VIOLET AND EOSIN. The Sheath was stained pink, and the cell-contents blue. × 1200.

The cell-wall may be either pliable or rigid. Pliability is observed in the long filaments, which are endowed with a slow vermicular movement, while rigidity accounts for the maintenance of the characteristic form of several species, such as spirilla.

Cell-contents. — The cell-protoplasm yields mycoprotein. In some it is homogeneous, and in others granular. The action of the aniline dyes indicates a close relation to nuclear protoplasm, though all nuclear stains are not suitable for bacteria. In some cases also, the bacteria remain stained under the influence of a reagent, which removes the colour from nuclei. The power of fixing the stain is not always present, and indicates a difference in the protoplasm of different species. Thus in staining phthisical sputum, the nitric acid removes the stain from all bacteria and bacilli

present, with the exception of the tubercle bacillus. This difference in the protoplasm of different species is also illustrated by the necessity in many cases of using special processes, owing to the ordinary methods being unsatisfactory or not producing any result.

The protoplasm of some bacteria contains starch granules; thus *Clostridium butyricum* gives the starch reaction with iodine. Sulphur granules are present in some species of Beggiatoa which thrive in sulphur springs. The colouring matter of the pigment bacteria is probably external to the cell as a rule; for example, in *Bacterium prodigiosum* the pigment granules are distinctly between the cells; on the other hand, in *Beggiatoa roseo-persicina*, or the peach-coloured bacterium, the special pigment *bacterio-purpurin* appears to be dissolved in the cell protoplasm. In *Bacillus pyocyaneus* the pigment is certainly not localised entirely in the cell; for it becomes rapidly diffused in the surrounding medium, considerably beyond the confines of the growth itself.

Gelatinous envelope.—In several species, either as a result of a secretion from the cell, or of the absorption of moisture and swelling up of the outer layer of the cell-wall, a mucinous or gelatinous envelope develops around them. This envelope may form a capsule, such as we meet with in certain bacteria found in the rusty sputum of pneumonia, and in *Micrococcus tetragonus*; or it may

occur as a continuous sheath around a chain of bacteria, which by its disappearance sets the individual links free. The capsule is soluble in water, and under some circumstances is difficult to demonstrate. In the pneumo-coccus of Friedländer the capsule disappears on cultivation, but reappears in preparations made from an inoculated animal. In the pleuritic fluid of a mouse these cocci are often found with a strikingly well-marked capsule, and in other capsuled cocci the extent of the envelope has been observed to vary considerably in the same species of bacterium.

When this gelatinous material forms a matrix, in which numbers of bacteria are congregated in an irregular mass, we have what is termed a zooglœa. The zooglœan stage is a resting stage, often preceded or followed by a motile stage. Thus bacteria may be present in a solution in an active state, and after a time a scum or pellicle forms on the surface of the liquid, which consists of zooglœa. At the edges of the zooglœa individuals may be seen again to become motile, detaching themselves from the edges of the mass, and swimming off in the surrounding fluid.

The same may be observed sometimes in cultivations started in nutrient gelatine. The inoculated bacteria grow and multiply, and liquefy the gelatine, and after a time a zooglœan film appears on the surface of the liquefied layer. On potatoes the appearances are very varied. In a bacillus which

readily develops on unsterilised potatoes, the zoogloœa may spread over the cut surface, forming a pellicle which can be raised *en masse* like a delicate veil. Another bacillus forms a zoogloœa, consisting of a tenacious layer which can be drawn out in long stringy threads. In *Ascococcus Billrothii* the gelatinous envelope develops to such an enormous extent that it forms the characteristic feature of the species (Fig. 46).

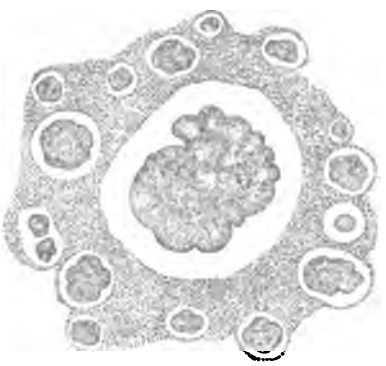


FIG. 46.—ASCOCOCCUS BILLROTHII, × 65. [After Cohn.]

Form.—The individual cells vary in form, and may either remain isolated or attached to each other. Round cells and egg-shaped cells are called *cocci*. The spherical form is the most common, but cocci are occasionally exclusively ovoid, as in *Streptococcus bombycis*. The giant cocci of some species are spoken of as *megacocci*, to distinguish them from the ordinary cocci, such as *micrococci*. The fission by which the cocci increase may take place in one direction only, and if the two resulting

cells remain attached to each other, they form a *diplococcus*. If these two cells again divide, and the resulting cells remain linked together, we get a chain or rosary, or *streptococcus* (Figs. 47, 48, 49).

FIG. 47.—STREPTOCOCCUS AND SARCINACOCCUS FROM A DROP-CULTIVATION, × 1200.

FIG. 48.—STREPTOCOCCUS IN THE BLOOD OF A RABBIT, × 1200.

These chains may consist of a few—four or five—individuals linked together, or of a far greater number, in which case the chains are generally curved or twisted. If the division occur in two

FIG. 49.—STREPTOCOCCUS OF PROGRESSIVE TISSUE NECROSIS IN MICE. [After Koch.]

directions, so that four cocci result, a tetrad or *merismopedia* is formed. If the division occur in three directions, one coccus divides into eight, and we get a packet form or *sarcinacoccus*. Immediately after division the daughter cells are not perfectly

circular, but are flattened or facetted where they are opposite to each other. They gradually become rounded off, and each daughter cell is then ready to divide in its turn. In other cases the cocci after division only form irregular heaps or collections like bunches of grapes. This form is sometimes distinguished as *staphylococcus*, but it cannot be considered an important feature. Where we find irregular masses or balls embedded in a copious gelatinous matrix, the extent of the latter affords a characteristic condition described as *ascococcus*.

Another type is the rod, characteristic of bacterium and bacillus. The rods may vary considerably in length. The very short rods with rounded ends are very difficult to distinguish from the oval cocci, but differ in that a rod, however short it may be, must have at least two sides parallel. The *vibrio* or bent rod may be considered as the connecting link between the rods and the corkscrew forms or *spirilla*. Lastly we have the filamentous forms, which may be straight, *leptothrix*, or wavy, *spirochæta* (Fig. 50), or the wavy thread may be looped and entwined on itself, *spirulina* (Plate I., Fig. 37).

By *involution* forms we signify certain irregular shapes which result especially in exhausted cultivations. They are peculiar, oval, pear-shaped, or irregular enlargements (Plate I, Figs. 31 to 36).

Movement.—Many bacteria are devoid of move-

ment throughout the whole of their life history. Others, during certain stages of their life cycle, and possibly some forms always, are endowed with locomotive power. The character of the movement is very varied, and ranges from a slow undulatory motion to one of extreme rapidity. Many appear to progress in a definite direction. Others move continuously, first in one direction and then in another, and others again seem to hesitate before altering their course. They may either glide along smoothly or progress with a tremulous action. They appear to be able to avoid obstacles, and to

FIG. 50.—SPIROCHÆTA FROM SEWAGE WATER, × 1200.

set themselves free from objects with which they have accidentally come into contact. Vibrios have a peculiar serpentine movement, but other forms, such as the commonly-known *Bacterium termo* and segments of spirilla, such as comma-bacilli, revolve around their long axis as well as make distinct progression. The comlpete spirilla are characterised by the familiar corkscrew movement. With regard to cocci there is some doubt as to whether they are endowed with independent movement; any quivering or oscillation is generally regarded as only brownian or molecular. In some

straight thread-forms, which are motile, the movement is very slow and vermicular in character, but in wavy threads, such as the *Spirochæte plicatilis*, there is not only an undulatory motion, with rapid progression across the field of the microscope, but if they are confined by more or less *débris*, they give very peculiar and characteristic spasmodic movements.

The rod-forms of *Proteus vulgaris* exhibit very extraordinary movements on the surface of solid nutrient gelatine. Groups of rods may be observed to pass each other in opposite directions. Single individuals meet and progress side by side, or one or more individuals may part from a group and glide away independently. Occasionally a number of rods progress in single file. It is, however, difficult to believe that these movements can occur on a solid surface. The author is inclined to believe that there is an almost inappreciable layer of liquid on the surface of the gelatine, which is expressed after the gelatine sets. In tubes of nutrient agar-agar gelatinised obliquely and then kept upright the liquid so expressed collects at the bottom of the sloping surface.

What the means are by which bacteria are endowed with the power of spontaneous movement and of progression may still be said to be unsettled. The author has watched the movement of long slender threads in sewage-contaminated water, which could only be explained by the inherent

contractility of the protoplasmic contents; for if any drawing or propelling organ existed in proportion to the length of the organism, it would probably have been visible. But in many cases the organism is undoubtedly provided with a vibratile

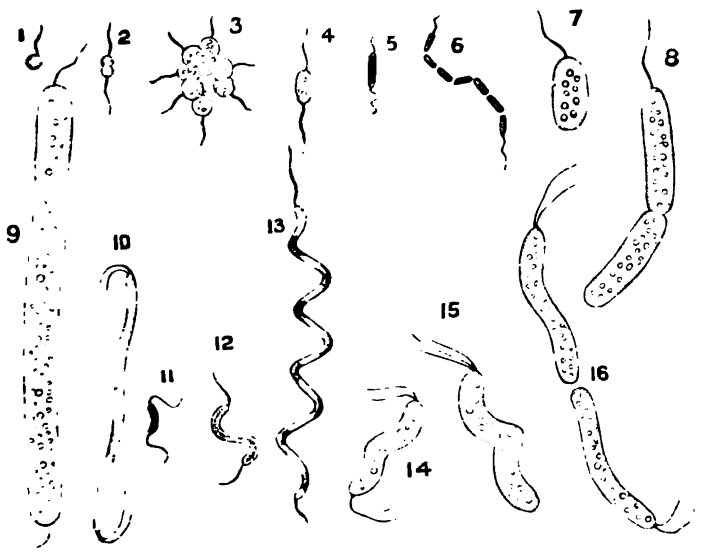


FIG. 51.

1. Coccus with flagellum. 2. Similar coccus dividing with two flagella. 3. Colony of flagellated macrococci of *Beggiatoa roseopersicina*. 4. Short rod from the same Beggiatoa with flagella [all after Zopf]. 5. Bacillus with flagella [from a photograph by Koch]. 6. *Bacillus subtilis* [after Brefeld]. 7, 8. Short rod-forms of *Beggiatoa roseopersicina* with one flagellum [after Zopf]. 9. Very long rod of the same, with flagellum at both ends [after Warming]. 10. Vibrio, with double flagellum at each end [after Warming]. 11. Vibrio, with flagella [from a photograph by the author]. 12. Spirillum with flagella [after a photograph by Koch]. 13. Spirillum with flagella [after Zopf]. 14. Spirillum with double flagella [after Zopt]. 15. *Beggiatoa roseopersicina* with a triple flagellum at one end; and 16. with a double flagellum at both ends [after Warming].

lash or flagellum at one end, or with one or more at both ends (Fig. 51).

Some observers believe that the movement of cocci is due to the existence of a flagellum. In *Bacterium termo* the existence of a lash at either end was first determined by the researches of Dallinger and Drysdale. In motile bacilli, such as the hay bacillus and *Bacillus ulna*, and in vibrios and spirilla, the flagella can be readily recognised by expert microscopists with the employment of the best lenses, and, what is of equal importance, proper illumination. They are objects of extreme delicacy, and tenuity, and in stained preparations may be absent from retraction or injury. Koch succeeded in photographing them after staining with logwood, which turned them a brown colour. They may also be stained with the aniline dyes, for the author has observed them in vibrios in preparations stained with gentian violet, from which also they have been photographed, in spite of the violet colour, by the use of isochromatic dry plates.

It is not certain whether the flagella are extensions of the cell-wall, or derived from the internal protoplasm. Van Tieghem holds the first view, and does not regard them as motile organs at all. Zopf, on the other hand, adheres to the second view, and moreover believes that they can be retracted within the cell-wall.

Reproduction.—Bacteria multiply by fission, and by processes which may be considered as representing fructification. The bacteria exhibiting the latter processes have been divided into two

groups, distinguished by the formation of endospores in the one, and of arthrospores in the other. In the process of fission the cell first increases in size, and a transverse septum forms from the cell-wall, dividing the internal protoplasm into two equal parts; these may separate and lead an independent existence, or remain linked together. In chains of

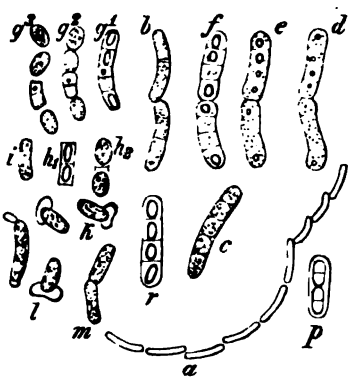


FIG. 52.—BACILLUS MEGATERIUM.

a. A chain of rods. × 250. The rest × 600.
b. Two active rods.
d to *f*. Successive stages of spore-formation.
h and *l*. Successive stages of germination.
[After De Bary.]

cocci the individual cells are easily visible and distinct, but in the thread-forms resulting from the linking together of rods, as in the anthrax bacillus, the composition of the thread is only demonstrated by the action of reagents.

Endospore formation may be conveniently studied in *Bacillus anthracis*, *Bacillus megaterium*, or *Bacillus subtilis*. The protoplasm becomes granular,

and at certain points in the thread a speck appears, which gradually enlarges and develops into a circular or egg-shaped, sharply defined, highly refractive body. The spore grows at the expense of the protoplasm of the cell, which in time, together with the cell-wall, entirely disappears, and the spore is set free. These phenomena are best seen in an immotile bacillus in a drop-cultivation

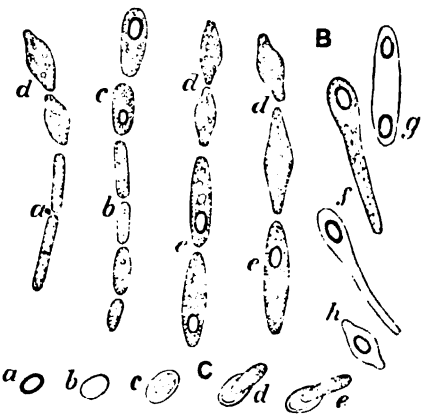


FIG. 53.—CLOSTRIDIUM BUTYRICUM, × 1020.
B. Stages of spore-formation.
C. Stages of germination.
[After Prazmowski.]

on a warm stage, the whole process may then be observed continuously from beginning to end. Spores may form in each link of the thread, so that a regular row results, or they may occur at irregular intervals. Spore-formation also occurs in free rods in the centre or at one end. Occasionally a spore develops at the extreme end, giving a bacillus the appearance of a drum-stick. The spore may be

considerably wider, but is never longer than the parent cell.

Arthro-spore formation is illustrated in *Leuconostoc mesenteroides*. Certain elements in the chain of cocci, apparently not differing from the rest,

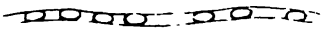


FIG. 54.—A THREAD OF BACILLUS ANTHRACIS WITH SPORES, IN A DROP-CULTIVATION, × 1400.

become larger, with tougher walls, and more refractive (Fig. 55). The remaining cells die, and these cells having acquired the properties of spores are set free, and can reproduce a new growth in any

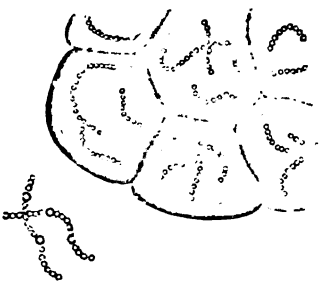


FIG. 55.—LEUCONOSTOC MESENTEROIDES; COCCI-CHAINS WITH ARTHROSPORES (after Van Tieghem and Cienkowski).

fresh nourishing soil. That this occurs in all species which do not form endospores is at present only a supposition.

Spores are invested by a thick membrane, which is believed to consist of two layers. To this they probably owe the property they possess of retaining vitality when desiccated, and of offering a greater

resistance to the action of chemical reagents and heat than the parent cells.

Spore-formation has been regarded by some as occurring when the nourishing soil is exhausted, thus providing for the perpetuation of the species. For instance, anthrax bacilli do not form spores in the living body, but when the animal dies it has been stated that development of spores takes place, and hence the danger of contaminating the soil if the body is disposed of by burial. Klein, however, has pointed out that if mice and guinea-pigs which have died of anthrax are kept unopened, the bacilli simply degenerate and ultimately disappear. Thus there is good reason for believing that spore-formation is not due to exhaustion of the pabulum, but probably free access to oxygen constitutes an important factor in inducing this condition. If we inoculate a potato with anthrax, copious spore-formation occurs, though we cannot consider that the nourishing soil has been exhausted. But we have in this case the surface of the potato freely exposed to the air in the damp-chamber. In the same way, in cultivations on agar-agar solidified obliquely, so as to get a large surface, spore-formation readily takes place. Contamination of a burial-ground must result, therefore, from bodies in which a post-mortem examination has been made, by which the blood and organs have been freely exposed to the air, or from animals which have not been examined, owing to their hides being soiled with

excretions, and with blood which issues from the mouth and nostrils before death.

When spores are introduced into a suitable medium at a favourable temperature, they develop again into rods. The spore loses its sharp contour, and, at one pole or on one side, a pale process bursts through the membrane, gradually growing into a rod from which the empty capsule is thrown off (Figs. 52 and 53).

Spores differ from the parent cells in their be-

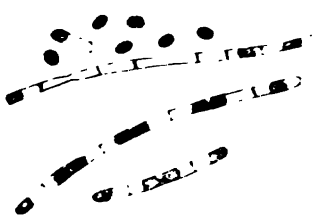


FIG. 56.—SPORES OF BACILLUS ANTHRACIS, STAINED WITH GENTIAN VIOLET, AFTER PASSING THE COVER-GLASS TWELVE TIMES THROUGH THE FLAME, × 1200.

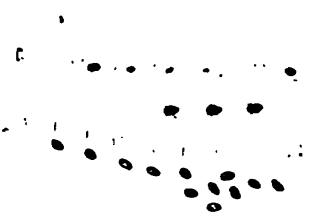


FIG. 57.—SPORE-BEARING THREADS OF BACILLUS ANTHRACIS, DOUBLE-STAINED WITH FUCHSINE AND METHYLENE BLUE, × 1200.

haviour to staining reagents. Like them, they can be stained with aniline dyes, but not by the ordinary processes. They require to be specially treated. This is probably due to the tough capsule, which must first be altered or softened by heat or strong acid, until it allows the stain to penetrate.

Once stained, they again differ from the parent cells in resisting decolorisation; this fact is taken advantage of to double-stain spore-bearing bacilli.

In staining micro-organisms, the protoplasm is

sometimes broken up into irregular segments or granules, as in many spirilla, and we may perhaps add the bacilli of tuberculosis and leprosy. The beaded appearance of the tubercle bacillus is well known. Some observers have regarded the beads, others the bright spaces between them, as spores. But spores in unstained preparations appear as glistening bodies with sharp contour, and do not stain at all, or very little, by the ordinary processes. It appears, therefore, very doubtful whether either the clear spaces or the beads are spores, especially as the tubercle bacillus, when unstained, is a slightly curved hyaline rod, without any differentiation into granules. These considerations led the author to stain and examine tubercular sputum from various sources under careful illumination, and with such lenses as Powell and Lealand's $\frac{1}{25}$ in. Hom. imm. The tubercle bacillus may then be frequently seen to consist of a very delicate sheath, holding together a number of deeply-stained granules, for the most part round or cylindrical, with irregular contour, and differing considerably in size, while the light interspaces are seen to vary in form according to the shape of the granules. In some preparations more distinct, and clearly ovoid, granules may be observed which are sometimes terminal. They can be readily demonstrated by taking a photograph with a $\frac{1}{25}$ in. Hom. imm., and subsequently enlarging the negative to from 2,500 (Fig. 58) to 6,000 diameters.

It is not impossible that these ovoid granules are spores, which, in their behaviour towards staining reagents, thus form an exception to the general rule. But there can be little doubt that a tubercle bacillus consists, for the most part, of a very delicate sheath, with protoplasmic contents which have a great tendency to be broken up or coagulated into little segments or roundish granules, owing possibly to the treatment they are subjected to in making a microscopical preparation. This, however, does not always occur, for the bacilli at times are not beaded, but are stained in their entirety. In the leprosy bacilli a similar appearance occurs. In stained sections the rods have a beaded appearance, but the intervals between the granules are sometimes very long, and occasionally the protoplasm appears to have collected only at the extreme ends of the rod (Fig. 59). Very probably the appearances in the case of the bacillus of glanders (Fig. 60), and the bacterium of chicken-cholera (Figs. 61 and 62) may be similarly explained.

FIG. 58.—TUBERCLE BACILLI IN SPUTUM, × 2500 (from photographs).

FIG. 59. LEPROSY BACILLI, FROM A SECTION OF SKIN, × 1200.

FIG. 60. GLANDERS BACILLI, FROM A SECTION OF A GLANDERS NODULE, × 1200.

The fact that tubercular sputum preserves its virulence for several months, even after desiccation, has been attributed to the formation of spores, and Babes has drawn attention to ovoid grains in old cultivations of the bacilli, which he succeeded in staining red, while the bacilli were stained blue.

FIG. 61.

BACTERIUM OF CHICKEN-CHOLERA, FROM BLOOD OF INFECTED HEN, × 1200.

In his definition of spirilla Zopf gives the spore-formation as absent or unknown. In comma-bacilli in sewage water, the author has often noted appearances very suggestive of refractive

FIG. 62.

BACTERIUM OF CHICKEN-CHOLERA, FROM MUSCLE JUICE OF AN INFECTED HEN, × 2500 [from a photograph].

FIG. 63.

COMMA BACILLI IN SEWAGE WATER, STAINED WITH GENTIAN VIOLET, × 1200.

spores (Fig. 63). The same also may be observed in vibrios, differing by their regular contour from the irregular spaces occasionally observed in stained preparations (Figs. 64 and 65). They are possibly only vacuoles.

Respiration and nutrition.—Like all a-chlorophyllous vegetables, bacteria require for their nutrition oxygen, nitrogen, carbon, water, and certain mineral salts. Many require free access to oxygen, others can derive it from the oxidised

compounds in the medium in which they grow. Pasteur divided bacteria into two great classes, the ærobic and anærobic; and considered that the latter not only had no need for oxygen, but that its presence was actually deleterious. Though this view must be considerably modified, the terms are convenient, and are still retained. They are well illustrated by the bacillus of anthrax, and the bacillus of malignant œdema; and a simple plan of demonstration has been employed by the author. A fragment of tissue from the spleen, for example,

FIG. 64.
VIBRIOS IN WATER CONTAMINATED WITH SEWAGE, × 1200.

FIG. 65.
SPIRILLUM UNDULA, × 1200.

known to contain anthrax bacilli, is deposited with a sterilised inoculating needle, with the necessary precautions, on the surface of nutrient agar-agar in a test-tube; another tube of nutrient agar-agar is liquefied, and when cooled down almost to the point of gelatinisation, a part is poured into the first tube, so that when it sets the piece of tissue is completely embedded. A piece of tissue from an animal suffering from malignant œdema is treated in the same way, and the tubes are placed in the incubator. If then we examine

them after two or three days, we shall find no change in the anthrax tube; the bacillus being eminently ærobic, no growth whatever has occurred. In the tube containing the bacilli of malignant œdema there will be a more or less characteristic cultivation.

The nitrogen which is essential for building up their protoplasm can be obtained either from albumins, or from ammonia and its derivatives. That the albumins can be dispensed with was shown by Pasteur, who employed an artificial nourishing solution built upon a formula representing the essential food constituents (p. 91).

Carbon is derived from such substances as cane sugar, milk sugar, and glycerine, and, in some cases, by the splitting up of complex proteid bodies.

Water is essential for their growth, but deprivation of water does not kill all bacteria. Desiccation on potato is employed for preserving some micro-organisms, as a new growth can be started, when required, by transferring some of the dried potato to fresh nourishing ground. Comma-bacilli, on the other hand, are destroyed by drying. Sugar, by abstracting water, prevents the development of micro-organisms in preserves.

Mineral or inorganic substances, such as compounds of sodium and potassium, and different phosphates and sulphates, are necessary in small proportions.

Circumstances affecting their growth. *Nature of the Soil.* — Though we know the elements necessary, we are, nevertheless, as yet unable to provide a pabulum suitable for all kinds of bacteria. Thus we are quite unable to cultivate some species artificially. Others will only grow upon blood-serum. Many grow upon nutrient gelatine; but some species only if it be acid or alkaline respectively. Whether in the latter case this is due purely to the reaction or to the presence of the particular ingredients is an unsettled point. Though the comma bacillus of Koch, like the majority of organisms, grows best on an alkaline medium, yet the surface of a potato is acid, and on this it is well known to flourish at the temperature of the blood.

Effect of temperature.—In their behaviour towards temperature bacteria vary considerably, but still for the majority we may distinguish a *maximum*, *optimum*, and *minimum* temperature.

Many grow best at the temperature of the blood, and hence the value of nutrient agar-agar, which is not liquefied at 37° C. The tubercle bacillus will only grow at a temperature varying between 30° and 41° C. On the other hand, many forms grow between the limits of 5° and 45° C. At these temperatures their functional activity is paralysed, but they are not destroyed, for by removal to favourable conditions they spring again to life. Bacteria seem to have a special power

of resisting the effects of cold. It has been stated that comma bacilli exposed to a temperature of —10° for an hour, and bacilli of anthrax after exposure to a temperature of —110° C., still retained their vitality. Temperatures over 50° to 60° C. destroy most bacteria, but not their spores; spores of anthrax retain their vitality after immersion in boiling water, but are destroyed by prolonged boiling. Roughly speaking, all pathogenic bacteria grow best at the temperature of the blood, and non-pathogenic bacteria at the ordinary temperature of the room.

Effect of movement. — Bacteria probably grow best when left undisturbed. Violent agitation of a vessel in which they are growing certainly retards their growth, but a steady movement is stated not to affect it; at any rate anthrax bacilli grow with enormous rapidity in the blood vessels, in spite of the circulation.

Effect of compressed air.—Paul Bert maintained that a pressure of twenty-three to twenty-four atmospheres stopped all development of putrefactive bacteria. Oxygen, under a pressure of five or six atmospheres, is stated to stop their growth. Other observers have, however, obtained different results.

Effect of gases.—Hydrogen and carbonic acid are stated to stop the movements of the motile bacteria. Chloroform is believed to arrest the changes brought about by the zymogenic species.

Electricity.—Cohn and Mendelsohn found that a constant galvanic current produced a deleterious effect owing to electrolysis. At the positive pole the liquid became distinctly acid, and at the negative pole distinctly alkaline. With a weak current there appeared to be no effect, two powerful cells at the very least being necessary.

Light.—Downes has shown that sunlight is fatal to putrefactive bacteria. This is believed to be due to a process of induced hyper-oxidation, from which living organisms ordinarily are shielded by protective developments of the cell-wall, or of colouring matter, which cut off injurious rays. Duclaux has investigated the same subject, and observed that micrococci were more sensitive to sunlight than the spore-bearing bacilli. Engelmann has described a bacterium whose movements cease in the dark, and Zopf states that in his cultures of *Beggiatoa roseo-persicina* the growth was much more strongly developed on the side of the vessel facing the light.

Chemical reagents.—Many substances, such as carbolic acid, corrosive sublimate, chlorine, bromine, etc., have a marked effect upon the growth of bacteria. This will be more fully described in another chapter. In several cases the bacteria themselves secrete a substance which is injurious to their future development.

Products of growth. — Bacteria may be grouped together according to the changes pro-

duced in the media in which they grow. Thus we have pigment-forming, fermentative, putrefactive, and pathogenic bacteria.

Chromogenic or pigment-forming bacteria elaborate during their growth definite colour stuffs. Such species are exemplified by *Bacillus ianthinus*, which produces a striking purple growth; *Bacillus pyocyaneus*, which secretes pyocyanin, a substance which has been isolated and obtained in a crystalline form; *Bacterium prodigiosum*, which produces a pigment allied to fuchsine; *Beggiatoa roseo-persicina*, which is characterised by the presence of bacterio-purpurin; *Sarcina lutea*, *Bacillus cyanogenus*, and many others.

Zymogenic or ferment bacteria produce their changes in non-nitrogenised media. *Bacterium aceti*, by its growth, produces the acetic fermentation in wine, by which alcohol taking up atmospheric oxygen is converted into vinegar—

$$C^2H^6O + O^2 = C^2H^4O^2 + H^2O.$$

The fermentation of urine, by which urea is converted into carbonate of ammonia, can be brought about by several micro-organisms, but notably by the *Bacterium ureæ*. The change produced is represented by the following formula:—

$$CO\begin{Bmatrix}NH^2\\NH^2\end{Bmatrix} + 2H^2O = \left(NH^4\right)^2CO^3.$$

Clostridium butyricum converts the salts of lactic acid into butyric acid, producing the butyric fer-

mentation in solutions of starch, dextrine, and sugar. These bacteria are agents in the ripening of cheese, and the production of sauerkraut. Thus, in a solution neutralised with calcium carbonate :—

$$2[Ca(C^3H^5O^3)^2]+H^2O=Ca(C^4H^7O^2)^2+CaCO^3+3CO^2+H^8.$$

In the so-called viscous fermentation the *Streptococcus viscosus* produces a gummy substance in wines. According to Pasteur, the change may be thus represented :—

$$25(C^{12}H^{22}O^{11})+25(H^2O)=12(C^{12}H^{20}O^{10})+24(C^6H^{14}O^6)+12(CO^2)+12(H^2O).$$

and as another example may be mentioned the *Bacillus acidi lactici*, through whose agency sugar of milk is converted into lactic acid :—

$$C^{12}H^{24}O^{12}=4(C^3H^6O^3).$$

Saprogenic or putrefactive bacteria play a most important *rôle* in the economy of nature. They produce changes allied to fermentation in complex organic substances. The nitrification of soil has been attributed to their agency. Their action on proteids, according to Hoppe-Seyler, may be compared to digestion; bodies like peptones are first produced, then leucin, tyrosin, and fatty acids; lastly indol, phenol, sulphuretted hydrogen, ammonia, carbonic acid, and water. They abstract the elements they require, and the remainder enter into new combinations. Associated with the formation of these substances are certain bodies, which have a poisonous effect when introduced into

animals. These poisonous alkaloids, *ptomaines*, produce a septic poisoning, which must be distinguished from septic infection. The effects of septic poisoning depend on the dose, whereas the effects of septic infection are, to a certain extent, independent of the dose. A small quantity of a septic poison may produce only transient effects, and a relatively large quantity may be necessary to produce vomiting, rigors, and death. Septic infection, on the other hand, may result equally from a small dose, because the poison introduced is a living organism which is capable of propagation and multiplication. Our knowledge of these alkaloids is greatly attributable to the researches of Selmi, Gautier, and also Brieger and others. Brieger has isolated ptomaines from the human cadaver, putrid meat, fish, and cheese. These substances—cadaverin, putrescin, saprin, peptotoxin, and many others—vary in their toxic properties.

Pathogenic bacteria are those which are genetically related to disease. Many organisms have been supposed to be pathogenic, or have been described in connection with diseases, which are only saprophytic associates. By the latter we mean organisms which feed upon dead organic matter. Such are many forms which are found on the skin, in the intestinal canal, and, according to Klein, in the liver and internal organs, where the tissues have lost their vitality, and the organisms, through some lesion, have been carried into the circulation.

That many organisms are causally related to disease, there is strong evidence in proof; for no organism can be considered to be productive of disease unless it fulfils the conditions which have been laid down by Koch (p. 2). Great stress must be laid upon the importance of successive cultivation through many generations, as the objection that a chemical virus may be carried over from the original source is thus overcome. Any hypothetical chemical poison carried over from one tube to another, would, after a great number of such cultivations, be diluted to such an immense extent as to be inappreciable and absolutely inert.

Though we may accept as a fact the existence of pathogenic organisms, we are not yet in a position to assert the means by which they produce their deleterious or fatal effects. Many theories have been propounded. It has been suggested that the organisms, micrococci for example, may be compared to an invading army. The tissue cells arrayed against them endeavour to assimilate and destroy them, but perish themselves in the attempt. This might explain the breaking down of tissue, and the formation of local lesions, but does not assist us in understanding the fatal result in thirty-six to forty-eight hours produced by the inoculation of the bacilli of anthrax. Another view is that the invading army seize upon the commissariat, appropriating the general pabulum, which

is so essential to the life of the tissues. But this would hardly account for so acute and fatal a result as anthrax, but would lead one to expect symptoms of inanition and gradual exhaustion. Moreover against this theory we have the fact that death may result, for example, from anthrax, with the occasional presence of comparatively few bacilli; and again, the blood may teem with parasites such as the flagellated monads in well-nourished, healthy-looking rats, without apparently causing any symptoms whatever. In the same category may be placed the theory that eminently ærobic organisms seize upon the oxygen of the blood and produce death by asphyxia. Another explanation is afforded by the suggestion of interference with the functions of the lung and kidney by mechanical blocking of the capillaries. Here the same objection is met with in the case of anthrax, the same fatal result may occur with only a few bacilli, while other cases yield very beautiful sections, looking like injected preparations from the mapping out of the capillaries with the countless crowds of bacilli (Plates XVI. and XVII.)

The most satisfactory explanation is probably afforded by analogy with the putrefactive bacteria. We have seen that they derive their necessary elements from complex organic substances, and accompanying the residue we find the presence of poisonous alkaloids. Do pathogenic bacteria act in the same way? Does the anthrax bacillus

produce a ptomaine *anthracin*, which in a certain dose produces death, independent of the number of bacilli, provided there are sufficient present to develop that dose? Though this is possible, observers as yet have failed to extract from cultivations in quantity of the anthrax bacillus any alkaloid with virulent properties.

Lastly it has been suggested that possibly a special ferment is secreted by the organisms, and that by the changes ultimately wrought by the action of this ferment, the symptoms and phenomena of disease arise. We have an analogy with this theory in the alkaline fermentation of urine by means of the *Torula ureæ*. By the researches of Musculus, and later of Sheridan Lea, it has been shown that a ferment is secreted by the cells which can be isolated in aqueous solution and is capable of rapidly inducing an active fermentation of urea. Either of the two last theories assists us in understanding how it is that in anthrax or in tuberculosis we may find the presence of only a few bacilli, or that, assuming both tetanus and hydrophobia to be due to microbes, we can have such a violent disturbance of the system produced by the presence of very few micro-organisms. We may conceive that different species of bacilli may vary greatly in their power of producing an alkaloid or secreting a ferment, just as the elaboration of pigment is much more marked in some species than in others; thus it need not follow that the number of

micro-organisms bears any relation to the virulence or activity of the substance they produce. There is, however, yet another factor in the production of disease. We know that in health we are proof against most of these micro-organisms; if it were not so, we should all rapidly fall victims to the tubercle bacillus or some others, which we in health inhale with impunity. We know that a microbe may only cause a local lesion in one animal, and death in another. It is still more striking that the same micro-organism, as is the case with anthrax, may have no effect whatever upon certain species of animals, though it is deadly to others. Again, an animal naturally susceptible to the effect of a pathogenic organism may be rendered proof against it. These matters will be discussed in a future chapter.

Distribution of Bacteria.—Bacteria are commonly described as ubiquitous. They are ever present in the air, though not in such exaggerated numbers as is commonly supposed. In nutrient media exposed to the air one is often astonished at times at the comparatively few bacteria which develop in comparison to the amount of floating matter, such as mineral particles, scales, spores of fungi, and *débris* known to be present. In water they are also present in considerable numbers, though of course varying according to the character of the water. Wherever there is putrefaction, they are present in vast numbers. In the soil, in

sewage, in the intestines; and in uncleanly persons especially, on the skin and between the teeth, various species may always be found, but in the healthy blood and healthy tissues bacteria are never present. In a previous chapter the method of examining the blood of living persons has been described, and there is, by this means, ample opportunity for satisfying oneself that bacteria are never to be found in the blood in health. The organs removed from a perfectly healthy animal, with the necessary precautions, into sterilised media can be kept indefinitely without undergoing putrefaction, or giving any development of bacteria. This has been established by many observers, notably Cheyne and Hauser; and the results of former observers to the contrary must be attributed to imperfect methods admitting of accidental contamination.

CHAPTER IX.

ANTISEPTICS AND DISINFECTANTS.

In the previous chapter several conditions were alluded to which affected the growth of bacteria, such as the nature of the nutrient soil, temperature, light, and electricity. The effect of certain chemical substances, and of excessive heat and cold, was also mentioned, but this constitutes a subject of such practical importance that it must be considered more fully.

Agents which retard the growth of bacteria are generally spoken of as *antiseptics* as distinguished from *disinfectants*, which altogether destroy their vitality.

Though chemical disinfectants, or germicides, when diluted, act as efficient antiseptics, the converse, that an antiseptic in a sufficiently concentrated form will act as a disinfectant, is not the case. The term "antiseptic," indeed, should be restricted to those substances or agents which arrest the changes bacteria produce, but which do not prevent their springing into activity when removed to favourable conditions. Thus excessive heat, which destroys

bacteria and their spores, is a true disinfectant; and excessive cold, which only benumbs them, retarding their development without killing them, is an antiseptic.

Spores have a greater power of resisting the action of these various agents than the parent cells, and many species of micro-organisms differ from each other in their resisting power. An exact knowledge of the subject can, therefore, only be based upon investigations which will determine the effect of these agents upon pure cultivations of the different micro-organisms causally related to putrefaction and disease. In the latter case, especially, this is not possible in the present state of our knowledge. In some cases of communicable disease there is considerable doubt as to the etiological importance of the organisms which have been described: in other cases no organisms have as yet been discovered, or the organisms cannot be artificially cultivated, or the disease is not reproduced by inoculation, so that there is no means of testing whether the agents have had any effect. One can, therefore, only draw general conclusions by selecting some well-known pathogenic and non-pathogenic micro-organisms, and considering the influence of chemicals, of hot air, and of steam upon them, as representing the effect upon the various contagia of disease and the causes of putrefaction.

Such knowledge must necessarily prove of the greatest importance,—to the sanitarian, who is con-

cerned in preventing the spreading of disease and in the disposal of putrefactive matter,—to the surgeon, who is anxious to exclude micro-organisms during surgical operations, and to arrest the development in wounds of bacteria which have already gained an entrance,—to the physician in the treatment of micro-parasitic diseases. The sanitarian and the surgeon must profit directly by such experiments, for in the disinfection of clothes and the sick-room by the one, and in the application of antiseptic dressings and lotions by the other, the micro-organisms are encountered as in the test experiments apart from the living body. The physician, on the other hand, is principally concerned in dealing with micro-parasites when circulating in the blood, or carrying on their destructive processes in the internal tissues. So far as our knowledge at present goes, the physician can avail himself but little of the effect of the direct application of the substances which have been found to retard or destroy the growth of the organisms in artificial cultivations, for the concentrated form in which they would have to be administered would prove as deleterious or as fatal to the host as to the parasites. Thus Koch has stated that to check the growth of the anthrax bacillus in man it would be necessary that there should be twelve grammes of iodine constantly in circulation; and that the dose of quinine necessary to destroy the spirilla of relapsing fever would be

from twelve to sixteen grammes. The retarding influence, however, of certain substances when diluted, and the fact that disinfectants are sometimes equally efficacious in a diluted form when their application is prolonged, seem to indicate measures which may be adopted, in some cases, with chances of success, such as the inhalation of antiseptic vapours in phthisis. For the most part the physician must look rather to combating the effects of micro-organisms by restoring to its normal standard the lowered vitality which enabled the bacteria to get a footing.

There is no wider field for research than the determination of the real effect of disinfectants and antiseptics. Painstaking and laborious as the researches are which have been hitherto made, the subject is so beset with fallacies, leading, in some cases, to totally erroneous conclusions, that it is not surprising that one meets on all sides with conflicting statements. The author has no intention of analysing these results, but a general idea will be given of the methods which have been employed, and for further details reference must be made to the original papers mentioned in the bibliography.

Chemical substances.—It was customary to judge of the power of a disinfectant or antiseptic by adding it to some putrescent liquid. A small portion of the latter was, after a time, transferred to some suitable nourishing medium, and the efficacy

of the substance estimated by the absence of cloudiness, odour, or other sign of development of bacteria in the inoculated fluid. Koch pointed out the errors that might arise in these experiments from accidental contamination, or from there being no evidence of the destruction of spores; and we are indebted to him for a complete and careful series of observations with more exact methods.

Instead of employing a mixture of bacteria, Koch's plan was to subject a pure cultivation of some well-known species with marked characteristics to the reagent to be tested. A small quantity was then transferred to fresh, nourishing soil, under favourable conditions, side by side with nutrient material inoculated from a cultivation without treatment with the disinfectant. The latter constituted a control test, which is most essential in all such experiments. To test the resistant power of bacteria which are easily destroyed, two species were selected, the so-called *Micrococcus prodigiosus*, and the bacillus of blue pus. These were cultivated on potatoes, the surface of which was sliced off and dried. A fragment transferred to freshly-prepared potato gave rise to a growth of the particular micro-organism; but if after treatment with some reagent no growth occurred, the conclusion was drawn that the agent was efficacious in destroying the vitality of the bacteria.

Anthrax bacilli in blood withdrawn from an animal just killed were taken to represent spore-

less bacteria, while silk threads steeped in an artificial cultivation of the bacilli and dried, afforded a means of testing the vitality of spores.

Even by employing pure cultivations on solid media, great precautions were necessary to avoid mistakes. If, for instance, a large quantity of the growth which had been subjected to some chemical solution were carried over to the fresh tube containing the nutrient medium, or if a silk thread, which had been dipped in a solution, were directly transferred to the new soil, enough of the supposed disinfectant might be mechanically carried over to retard the development of the bacteria, though it was ineffectual in destroying them. From a growth not appearing, the conclusion might be drawn that the spores or the bacteria had been affected, and so a mistake occurs. To avoid this Koch made a point of transferring a minimum of the disinfected growth to as large a cultivation area as possible, so that any chemical substance mechanically carried over, would be so diluted as to be inert. For the same reason threads, after withdrawal from the disinfecting solution, were rinsed in sterilised water, or weak alcohol, and then transplanted; or, instead of judging from the development on nutrient gelatine, the effect of inoculation in a healthy animal was made the test.

A few examples may be quoted in illustration. Silk threads, impregnated with anthrax spores, were placed in bottles containing carbolic acid of

various strengths. A thread was removed from each on successive days, and transferred to nutrient gelatine, and the result noted. It was found that immersion of the thread in a 5 per cent. solution of carbolic acid was sufficient in two days to effect complete sterilisation, and seven days in a 3 per cent. solution was equally efficacious. Since for practical purposes a strength should be selected which would be effectual in twenty-four hours, Koch recommended that for general use, allowing for deterioration by keeping, a solution containing not less than 5 per cent. should be employed, and for complex fluids probably a still higher percentage would be necessary. In the case of sporeless bacilli the results were very different. Blood, containing the bacilli, from an animal just killed, was dried on threads, and after exposure for two minutes to a 1 per cent. solution, was completely sterilised. Fresh blood mixed with a 1 per cent. carbolic solution produced no effect on inoculation. If, on the other hand, the blood was mixed with a ·5 per cent. solution, the virulence was not destroyed. The facility with which the bacilli are destroyed, compared with their spores, illustrates how easily errors may occur, if mere arrest of growth or loss of motility be regarded as a sign of the efficacy of disinfection.

To test vapours, Koch exposed anthrax spores or the spores which occur in garden earth by suspending them over solutions, *e.g.*, of bromine or

chlorine in a closed vessel. After a time they were transferred to a nutrient medium to test their vitality. To test the power of sulphurous acid gas, the spores were spread about in a room in which the gas was generated by burning sulphur in the ordinary way for disinfecting a room. To test chemicals which might be recommended for disinfecting vans and railway carriages, spores were laid on boards which were then washed or sprayed, and the spores then transferred to the nutrient gelatine.

By such simple methods Koch investigated a long list of chemical reagents, and according to these experiments the salts of mercury, and the chloride especially, proved most valuable. Where heat is not admissible, these compounds were therefore highly recommended, though their poisonous nature is a drawback to their indiscriminate use. Koch states, for disinfecting a ship's bilge, where a 5 per cent. solution of carbolic acid must be left for forty-eight hours, a 1 in 1000 solution of mercuric chloride would only require a few minutes.

There is, on the other hand, reason for doubting the efficacy of mercuric chloride; for, though anthrax spores subjected to a 1 in 20,000 solution of mercuric chloride for ten minutes, and then washed in alcohol, gave no growth in nutrient gelatine, silk threads exposed for ten minutes to a 1 in 20,000 solution, or even 1 in 10,000, still proved fatal to mice.

Herroun considers that the value of mercuric chloride as an antiseptic is much over-rated, as he has cultivated ordinary septic bacteria in albuminous filtrates, containing 1 in 2,000. It is precipitated by albumins if used of greater strength, and is readily converted by the sulphur of albuminous bodies into mercuric sulphide,—a compound which has practically no antiseptic properties.

Sternberg has also made an elaborate series of experiments with regard to the action of germicides. In this case cultivations of well-known pathogenic organisms in liquid media were employed. The supposed germicide was added to the liquid cultivation, and after two hours a fresh flask of sterilised culture was inoculated from the disinfected cultivation, and placed in the incubator. In twenty-four to forty-eight hours, if the chemical was not efficient, there was evidence of a growth of bacteria. Blyth has investigated the disinfection of cultivations of *Bacterium termo*, of sewage, and typhoid excreta, and, in conjunction with Klein, the effect of well-known disinfectant materials on anthrax spores. Miquel, Laws, and others, have also contributed to our knowledge of the effect of antiseptics and disinfectants upon micro-organisms. In spite of all that has been done, there is room for many workers; a great deal of ground must be gone over again to rectify discrepancies, examine conflicting results, and thus determine what

observations may be relied upon for practical application.

Hot Air and Steam.—Koch, in conjunction with Wolfhügel, also tested the value of hot air. A similar plan was adopted as in disinfection with chemicals. Bacteria and spores were subjected for a certain time to a known temperature in a hot-air chamber, and then were transferred to a nourishing soil, or animals were inoculated.

Paper parcels, blankets, bags, and pillows, containing samples of micro-organisms wrapped up inside, were also placed in the hot-air chamber, to test the power of penetration of heat.

The conclusions from such experiments were as follows:—

Sporeless micro-organisms at a little over 100° C. are destroyed in one hour and a half.

Spores of bacilli require three hours at 140° C.

If enclosed in pillows and blankets, exposure from three to four hours to 140° C. is necessary.

Spores of fungi require one and a half hours at 110—115° C.

Further experiments showed that at the temperature necessary for the destruction of spores of bacilli almost all fabrics are more or less injured.

Koch, in conjunction with Gaffky and Löffler, also investigated the effect of steam under pressure and at the atmospheric pressure.

Rolls of flannel with anthrax spores or earth spores, and a thermometer wrapped up inside, were

subjected to steam, and the results compared with the effect obtained with hot air.

Thus in hot air four hours' exposure to a temperature of 130° C.—140° C. brought the temperature inside the roll to 85° C., and the spores were not injured; on the other hand, exposure to steam under pressure at 120° C. for one and a half hours, raised the internal temperature to 117° C. and killed the spores.

By such experiments the superior penetrative power of steam-heat was established.

To test steam-heat at the atmospheric pressure, water was boiled in a glass flask with its neck prolonged by means of a glass tube, the temperature in which was found to be uniform throughout. Anthrax and earth spores placed in the tube were found to be unable to withstand steam at 100° C. even for a few minutes. It was, therefore, concluded that disinfection by steam at atmospheric pressure was superior to hot air from its greater efficiency, and to steam under pressure from the simplicity of the necessary apparatus.

Parsons and Klein made some experiments which were more in favour of dry heat than the above. These observers state that anthrax bacilli are destroyed by an exposure of five minutes to from 100° C. to 103° C., and that anthrax spores are destroyed in four hours at 104° C., or in one hour at 118°C. Guinea-pigs inoculated with tuberculous pus which had been exposed for five minutes to 104° C.,

remained unaffected. They concluded that as none of the infectious diseases, for which disinfecting measures are in practice commonly applied, are known to depend upon the presence of bacilli in a spore-bearing condition, their contagia are not likely to retain their activity after being heated for an hour to 105° C. (220° F.).

In experiments with steam, the results were in accordance with those already given, and complete penetration of an object by steam-heat for more than five minutes was deemed sufficient. They also arrived at the same result as in Koch's experiments, that steam-chambers are preferable to those in which dry heat is employed, though it must be borne in mind that some articles, such as leather, are injured by exposure to steam.

CHAPTER X.

IMMUNITY.

THE condition of being insusceptible to an infective disease may be either natural or acquired. In the description of the pathogenic organisms several examples of natural immunity will be encountered. The bacillus of septicæmia, so fatal to house mice, has been shown to have no effect upon field mice. The bacillus of anthrax is innocuous to pigs, cats, white rats, and to adult dogs, asses, and horses. The bacterium of rabbit septicæmia is equally inert in dogs, rats, and guinea-pigs. The immunity may be as in these cases complete, or only partial. Ordinary sheep are very easily affected with anthrax, but Algerian sheep only succumb to large doses of the virus. Natural immunity may not only be characteristic of certain species, but it may occur in certain individuals of a susceptible species. The same occurs in man, for certain individuals, though equally exposed during an epidemic of small-pox, may escape where others readily fall victims to the disease.

Acquired immunity is illustrated by the protection afforded by one attack of the exanthemata against subsequent attacks. Thus one attack of measles or small-pox, as a rule, affords complete protection. A knowledge of the immunity resulting in the latter case led to the introduction of inoculation of small-pox prior to the establishment by Jenner of the protective influence of vaccination.

Immunity may be acquired by acclimatization, for the inhabitants of tropical climates are less susceptible to the diseases of the country, malarial fevers for instance, than strangers.

In civilised communities also there appears to be a degree of acquired immunity, for the infectious diseases introduced among savages or isolated communities have assumed the most virulent properties.

The immunity acquired by protective inoculation constitutes, in connection with the study of pathogenic micro-organisms, a subject of pre-eminent interest and importance. Pasteur, in his researches upon fowl-cholera, observed that after non-fatal cases the disease either did not recur, or the severity of a subsequent attack was in inverse proportion to the severity of the first attack. It occurred to him to endeavour to obtain the virus of this disease in a form which would provoke a mild attack of the disease, and thus give protection against the virulent form. This attenuation or mitigation of the virus was

successfully attained in the following manner: Cultivations of the microbe, in chicken-broth, were allowed to remain with a lapse of several months between the carrying on of successive cultivations in fresh media. The new generations which were then obtained were found to have diminished in virulence, and ultimately a virus was obtained which produced only a slight disorder; on recovery the animal was found to be proof against inoculation with virulent matter. The explanation given by Pasteur of this change was, that prolonged contact with the oxygen of the air was the influence which diminished the virulence, and he endeavoured to prove this by showing that if broth were inoculated in tubes which could be sealed up, so that only a small quantity of air was accessible to the microbe, the virulence of the cultures was retained.

Toussaint investigated the possibility of attenuating the virus of anthrax. Sheep injected with 3 ccm. of defibrinated blood, containing anthrax bacilli, which had been exposed to 55° C. for ten minutes, recovered, and were afterwards insusceptible. Pasteur subsequently argued that this method did not admit of practical application; difficulties would arise in dealing with infective blood in quantity, and artificial cultivations started from this blood could not be relied upon, as they proved sometimes as virulent as ever.

Pasteur endeavoured to apply the same method

for obtaining an attenuated virus of anthrax, as he had successfully employed in chicken-cholera. A difficulty was soon encountered, for in cultivations of the bacillus with free access of air spore-formation readily takes place, and the spores are well known to have an extraordinary power of retaining their virulence. Pasteur found that the bacilli ceased to develop at 45° C., and he believed that spore-formation ceased at 42°—43° C., the bacilli continuing to develop by fission only. The cultivations were, therefore, kept at this temperature, and at the end of eight days the bacilli were found to have lost their virulence, and were quite inert when inoculated in guinea-pigs, sheep, or rabbits. This total destruction was, however, preceded by a gradual mitigation, so that a virus could be obtained, by taking it at the right time, which only gave a mild disease, and afforded subsequent protection.

At Melun, in 1881, the protective inoculation against anthrax was put to a practical test. Sheep and oxen were inoculated with the mitigated virus, and then with a virulent form; at the same time other sheep and oxen were inoculated with the virulent form without previous vaccination as a control experiment. The unprotected sheep died without exception; the unprotected oxen suffered from œdematous swellings at the seat of inoculation, and a rise of temperature; but all the protected animals remained healthy.

As a result of these experiments an idea arose that by preventive inoculation with attenuated virus all communicable diseases would in time be eradicated; but this does not follow, for all communicable diseases do not confer immunity after a first attack, and in some cases the very reverse is believed to occur. Thus erysipelas of the face leads to an increased liability to subsequent attacks of the same disease. Again, the occurrence of one disease is stated to induce a liability to others; small-pox and typhoid fever are regarded as predisposing to tuberculosis; so that the principle of preventive inoculation does not apply in these cases, and its effect would probably tend rather to deleterious results than otherwise. Even with regard to the prevention of anthrax, Pasteur's researches were opposed and criticised. Koch investigated the subject, and came to the conclusion that the process did not admit of practical application, chiefly on the ground that as immunity only lasted a year, the losses from the vaccination process would be as great or even greater than from the spontaneous disease; further, there was danger in disseminating a vaccine of the strength required to be effectual. Chauveau proved that the attenuation was due to the temperature, and not to the prolonged effect of oxygen. By keeping cultivations at 42°—43° C. *in vacuo*, the virulence was found to disappear in twenty-four hours, and by keeping cultivations at a low temperature with free

access of air the virulence was retained. Chauveau considered, therefore, not only that oxygen was not the agent, but that the mitigation was much more easily effected in its absence. In spite of these adverse criticisms, these researches nevertheless confirmed the principle of Pasteur's conclusion, that immunity could be induced by experimental measures, and further showed that he had considerably advanced the method by which this could be effected.

Chauveau succeeded also in attenuating the virus by a modification of Toussaint's method. Sterilised broth was inoculated with the bacilli, and placed in the incubator at 42°—43° C. After the lapse of twenty hours it was removed to another incubator at 47° C. According to the time of exposure to this increased temperature, the mitigation varied in degree. Thus inoculation with the virus, before it was exposed to 47° C., was fatal to guinea-pigs; but after one hour at 47° C. the virulence was diminished, and, though ultimately fatal, life was prolonged; after two hours' exposure at 47° C. only half the animals died; and after three hours' exposure they recovered and were rendered refractory to subsequent inoculation.

Attenuation of the virus has also been induced by chemical means. Chamberland and Roux stated that a fresh growth started from a cultivation of bacilli which had been subjected for twenty-nine days to $\frac{1}{600}$ of carbolic acid was found to

be inert in guinea-pigs and rabbits. Bichromate of potash added to a cultivation in the proportion of $\frac{1}{12000} - \frac{1}{5000}$ gave, after three days, a new growth, which killed rabbits, guinea-pigs, and half the sheep inoculated; after ten days, rabbits and guinea-pigs, but not sheep; and after a longer time even guinea-pigs were unaffected.

In other diseases similar results have been obtained.

Arloing, Cornevin, and Thomas found that by inoculating a small quantity of the virus of symptomatic anthrax anywhere in the subcutaneous connective tissue, or a moderate quantity at the root of the tail, and even by intravenous injection, immunity was obtained from a virulent dose.

In swine-erysipelas, Pasteur and Thuillier obtained attenuated virus upon quite another principle. They discovered that by passing the virus through pigeons the virulence was increased, but by passing it through rabbits it was progressively diminished. Thus a virus was obtained from the rabbit, which produced only a mild disease in pigs, and after recovery complete immunity. Similarly in rabies Pasteur finds that passage of the virus through various animals considerably modifies its properties. By inoculating a monkey from a rabid dog, and then passing the virus through other monkeys, the virulence is diminished; but by inoculating a rabbit from the dog, and passing the virus from rabbit to rabbit, the virulence is increased. More recently

Pasteur has employed another method of attenuating the virus of rabies. The spinal cord of inoculated rabbits is removed with all possible precautions, and portions a few centimetres in length are suspended in flasks in which the air is dried by fragments of potash. By this process the virulence is found to gradually diminish and finally disappear.

Animals inoculated with portions of these cords, after suspension for a certain time, are rendered refractory to inoculation with virulent cords. Having rendered dogs, which had been previously bitten, free from the supervention of symptoms of hydrophobia by means of protective inoculation, Pasteur proceeded to apply the same treatment to persons bitten by rabid animals, with results which tend to the belief that a prophylactic for rabies has been found, though this must still be considered to be *sub judice*.

The question as to what constitutes immunity is a vexed one.

Raulin has shown that *Aspergillus niger* develops a substance which is prejudicial to its own growth in the absence of iron salts in the nutrient soil. Pasteur has suggested that in rabies side by side with the living and organised substance there is some other substance which has, as in Raulin's experiment, the power of arresting the growth of the first substance. If we accept the theory of arrest by some chemical substance, we must suppose that in the acquired immunity afforded by

one attack of an infectious disease this chemical substance is secreted, and, remaining in the system, opposes the onset of the micro-organism at a future time. In the natural immunity of certain species and individuals we must suppose that this chemical substance is normally present.

Another theory is, that the micro-organisms assimilate the elements which they require for their nutrition from the blood and tissues, and render the soil impoverished or otherwise unsuitable for the development of the same micro-organisms hereafter; this condition may be permanent, or the chemical constitution of the tissues may be restored to normal, when immunity ceases. If, however, we explain acquired immunity by the result of the growth of a previous invasion of micro-organisms, we are still confronted with the difficulty of explaining natural immunity.

A third theory is that the tissues are endowed with some power of vital resistance to the development of micro-organisms, similar to the vital resistance to the coagulation of the blood, which is supposed to exist in the lining membrane of the healthy blood-vessel; that in some species and individuals this exists to a high degree, and hence their natural immunity But this does not explain how one attack confers immunity from a subsequent one. One would expect that the vital resistance would invariably be lowered by a previous attack, and increased liability be the constant result.

Lastly, that leucocytes appear to have the power of destroying bacteria in some cases, has been demonstrated by the researches of Metschnikoff.

If anthrax bacilli are inoculated in the frog, the white blood-cells are observed to incorporate and destroy them until they entirely disappear, and the animal is not affected. But if the animal, after inoculation, is kept at a high temperature, the bacilli increase so rapidly that they gain the upper hand over the leucocytes, and the animal succumbs.

In septicæmia of mice the white blood-cells are attacked and disintegrated by the bacilli in a similar way. It is difficult, however, to accept any explanation of immunity from these observations, —to suppose, for example, that immunity depends upon the micro-organisms being unable to cope with the leucocytes in certain species. It is difficult to conceive that the leucocytes in the blood and tissues in the field mouse are differently constituted from those in the house mouse, so that they form an effectual barrier in the one case, though so readily destroyed in the other.

PART III.

SYSTEMATIC AND DESCRIPTIVE, WITH SPECIAL MICROSCOPICAL METHODS.

CHAPTER XI.

CLASSIFICATION OF BACTERIA.

LEEUWENHOECK,* two hundred years ago, recognised, and described, microscopic organisms in putrid water and saliva, which probably correspond with organisms, such as vibrios and leptothrix of modern times. During two centuries these minute beings have afforded histologists a subject for controversy and dispute. Existing as they do upon the very borderland of the vegetable and animal kingdoms, not only have they been transferred from one to the other, but even the question has been raised whether the smaller forms should be considered as living beings at all.

In reviewing the history of the various classifications which have from time to time been proposed, we shall see that the gradual improvements in the means of studying such minute objects, the methods of cultivating them artificially, and of studying their chemistry and physiology, and the ever-increasing revelations of the microscope, have resulted in

* Leeuwenhoeck, *Op. Omnia* (Lugd. Batav., 1722).

establishing these microscopic objects as members of the vegetable kingdom, ranking among the lowest forms of fungi. While enabling us to settle their position as a whole, these improved methods have further given us so great an insight into the life-history of individual forms, that, with regard to the division into genera and species, we are up to the present time still in a position of doubt and uncertainty.

Müller, in 1773, was the first to suggest a classification. He established two genera, *Monas* and *Vibrio*, and grouped them with the *Infusoria*. In 1824 Bory de Saint Vincent also attempted a classification; but it was not until Ehrenberg in 1838, and Dujardin in 1841, worked at the subject, that a scientific distinction of species was attempted.

Ehrenberg described four genera:—

I. Bacterium .	. filaments straight, rigid.
II. Vibrio .	. filaments snake-like, flexible.
III. Spirillum .	. filaments spiral, rigid.
IV. Spirochæte .	. filaments spiral, flexible.

Dujardin united *Spirillum* and *Spirochæte*, and classed them thus:—

I. Bacterium .	filaments rigid, vacillating.
II. Vibrio .	. filaments flexible, undulatory.
III. Spirillum.	. filaments spiral, rotatory.

Up to that time bacteria were still considered as

Infusoria; but the year 1853 marked the commencement of a new era in their history, for Robin then pointed out the affinity of the *Bacteria* and *Vibrios* to *Leptothrix.* Davaine, in 1859, still more definitely insisted that the *Vibrios* were vegetables, and that they were in fact allied to the *Algæ.*

Since that time a flood of light has poured in upon the subject through the writings of Hoffmann, Pasteur, Cohn, Rabenhorst, Hallier, Billroth, Warming, Nägeli, Magnin, Marchand, Sternberg, Van Tieghem, Lister, Klein, Koch, Flügge, De Bary, Zopf, Cornil, Babes, and many other workers in the recent widespread revival of bacteriological research.

Of all these writers we are most indebted to Cohn,* not only on account of his researches, which extended over very many years, but also for his system of classification, which has since been almost universally adopted.

In his first classification, published in 1872, Cohn considered the Bacteria as a distinct group belonging to the *Algæ*, and divisible into four tribes, including six genera:—

I. Sphærobacteria	globules (Micrococcus).
II. Microbacteria .	short rods (Bacterium).
III. Desmobacteria .	long rods (Bacillus and Vibrio).
IV. Spirobacteria .	spirals (Spirochæte and Spirillum).

* Cohn, *Beiträge zur Biologie der Pflanzen*, 1872, *et seq.*

Cohn noted, in spite of placing them with the *Algæ*, that the absence of chlorophyll connected the *Bacteria* to *Fungi*, and we find Nägeli subsequently adopting this view, and employing the term *Schizomycetes*.

Billroth, in 1874, disputed the division into species, and considered that all the forms described by Cohn were but developmental forms of one micro-organism, *Coccobacteria septica*. In the following year Cohn answered the criticism of Billroth, and produced a second classification, in which he still maintained that distinct genera and species existed. The genera Cohn considered to be distinguished by definite differences in shape, which were adhered to throughout life, while some special feature, as a difference in size or physiological action, or some minute difference in form, determined the various species. Cohn illustrated, by his well-known comparison of a sweet and a bitter almond the appearances of which are similar but the properties very different, that a distinction into species might depend upon a difference in physiological action only. Others strongly support Cohn's views. By cultivating various micro-organisms through several generations, many conclude that a micrococcus cannot be transformed into a bacterium, or a bacterium into a bacillus or spirillum. Koch does not believe—and in this he is supported by Klein—that a bacillus can change its nature, and be converted

from a harmless into a pathogenic form, as asserted by Büchner.*

The second classification of Cohn (1875) only differed from the first in that, instead of keeping the bacteria as a separate group, he placed them, from their close relationship with the *Phycochromaceæ*, under a new group, the *Schizophytes*, and added the genera *Leptothrix*, *Beggiatoa*, *Crenothrix*, *Sarcina*, *Ascococcus*, *Streptococcus*, *Myconostoc*, and *Streptothrix*.

Nägeli maintained that *Bacteria* were allied to *Yeasts*, and should be included in the class of *Fungi*. In fact, he divided the *fungi producing decomposition* into :—

Mucorini	moulds
Saccharomycetes	yeasts
Schizomycetes	fission-fungi

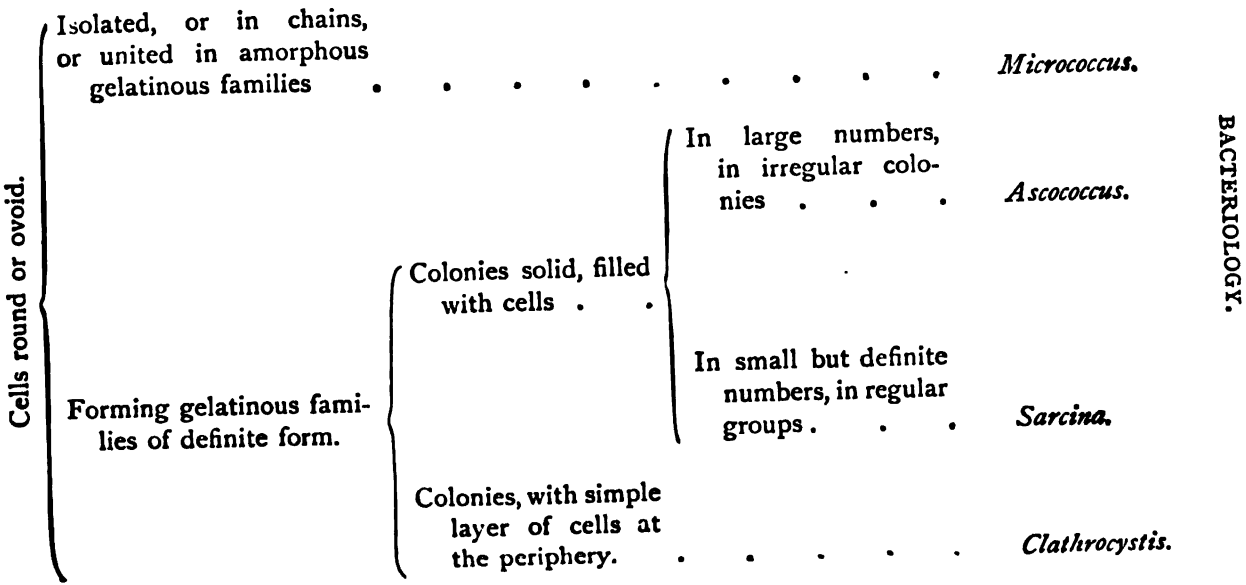
(This last class comprising bacteria.)

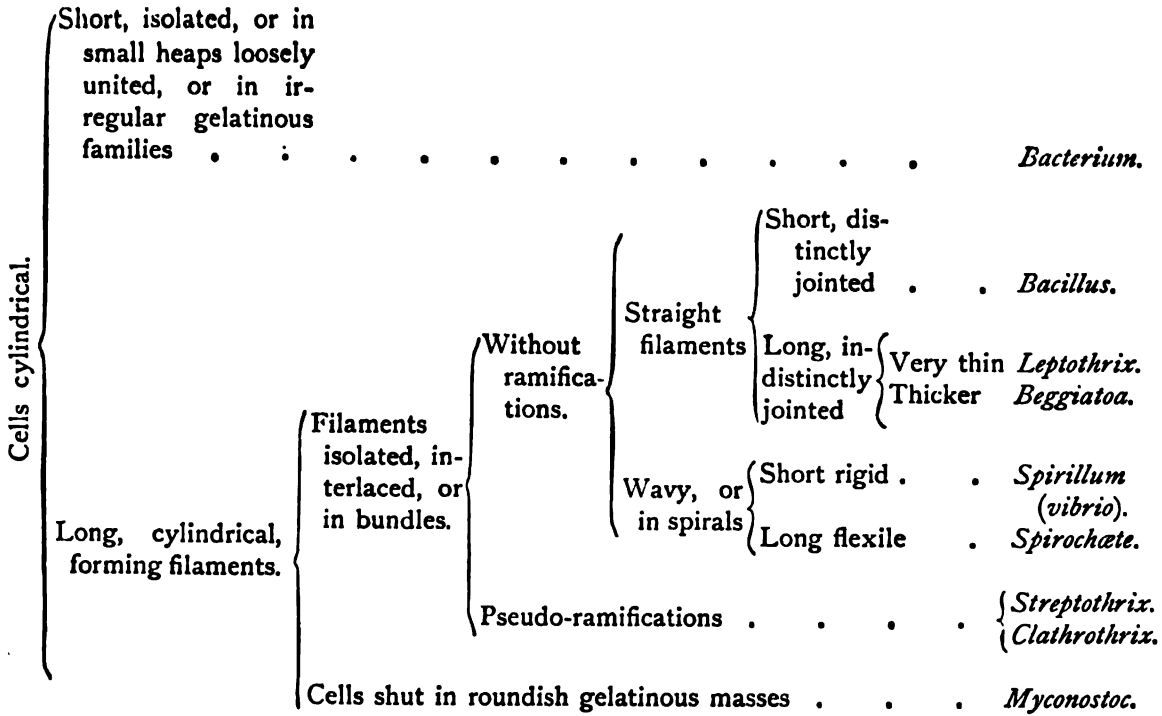
Flügge,† following Rabenhorst, maintained the term *Schizomycetes*, and divided them as follows :—

* Büchner, *Ueber d. experim. Erzeugung d. Milzbrandcontagiums aus d. Heupilzen.*

† Flügge, *Fermente und Mikroparasiten.* 1883.

SCHIZOMYCETES (FLÜGGE'S ORIGINAL CLASSIFICATION).


Cells round or ovoid.
Isolated, or in chains, or united in amorphous gelatinous families . . . . . *Micrococcus.*
Forming gelatinous families of definite form.
Colonies solid, filled with cells . .
In large numbers, in irregular colonies . . . *Ascococcus.*
In small but definite numbers, in regular groups . . . *Sarcina.*
Colonies, with simple layer of cells at the periphery. . . . . *Clathrocystis.*


Cells cylindrical.
Short, isolated, or in small heaps loosely united, or in irregular gelatinous families . . . . . Bacterium.
Long, cylindrical, forming filaments.
Filaments isolated, interlaced, or in bundles.
Without ramifications.
Straight filaments
Short, distinctly jointed . . Bacillus.
Long, indistinctly jointed
Very thin Leptothrix.
Thicker Beggiatoa.
Wavy, or in spirals
Short rigid . . Spirillum (vibrio).
Long flexile . Spirochæte.
Pseudo-ramifications . . . . Streptothrix. Clathrothrix.
Cells shut in roundish gelatinous masses . . . Myconostoc.

The belief is nevertheless rapidly gaining ground that the lowest forms of vegetable life cannot be divided by a hard and fast line into a series with chlorophyll (*Algæ*), and a series without it (*Fungi*), and the tendency now is to solve the difference of opinion between Cohn and Nägeli by following the example of Sachs, and amalgamating the two series into one group, the *Thallophytes*.

Researches by competent observers have quite recently clearly demonstrated that several micro-organisms in their life cycle exhibit successively the shapes characteristic of the orders of Cohn.

This doctrine of *pleomorphism*, now widely accepted, was distinctly foreshadowed in a publication* by Lister in 1873, though this memoir contained certain conclusions which have since been abandoned. Lister described forms of cocci, bacteria, bacilli, and streptothrix in milk, which he regarded as phases of the same micro-organism, *Bacterium lactis*. As a result of his observations Lister remarks that "any classification of bacteria hitherto made from that of Ehrenberg to that of Cohn based upon absolute morphological characters is entirely untrustworthy." To Lankester, however, belongs the credit of having definitely and precisely formulated this doctrine. In a paper,† also published in 1873, Lankester observed that the series of form-phases which he had discovered in the case of a

* Lister, *Quart. Journ. Microscop. Sci.*, 1873, pp. 380—408.
† Lankester, *Ibid*, pp. 408—425, and 1876, pp. 278—283.

peach-coloured bacterium led him to suppose that the natural species of these plants were "within the proper limits protean, and that the existence of true species of bacteria must be characterised, not by the simple form-features used by Cohn, but by the ensemble of their morphological and physiological properties as exhibited in their complete life-histories." Lankester inferred that these phase-forms were genetically connected from their all possessing the common characteristic of a special pigment bacterio-purpurin. These conclusions were vigorously opposed by Cohn, and doubt still remains in the minds of some as to whether the different forms are really only stages in the life-history of a single species. Nevertheless the theory of pleomorphism has steadily gained ground ever since.

Among the recent observers Cienkowski and Neelsen have worked out the different forms assumed by the *bacillus of blue milk;* Zopf has in a similar manner investigated *Cladothrix*, *Beggiatoa*, and *Crenothrix*, and traced out various forms (Fig. 66); Van Tieghem has investigated *Bacillus amylobacter* with a similar result; Hauser has quite recently described bacillar, spirillar, and spirulinar, and various other forms in the *Proteus mirabilis* and *Proteus vulgaris*. These facts obviously shake the very foundation of Cohn's classification, and we are left without possessing a sound basis for classification into genera or species. The mode of repro-

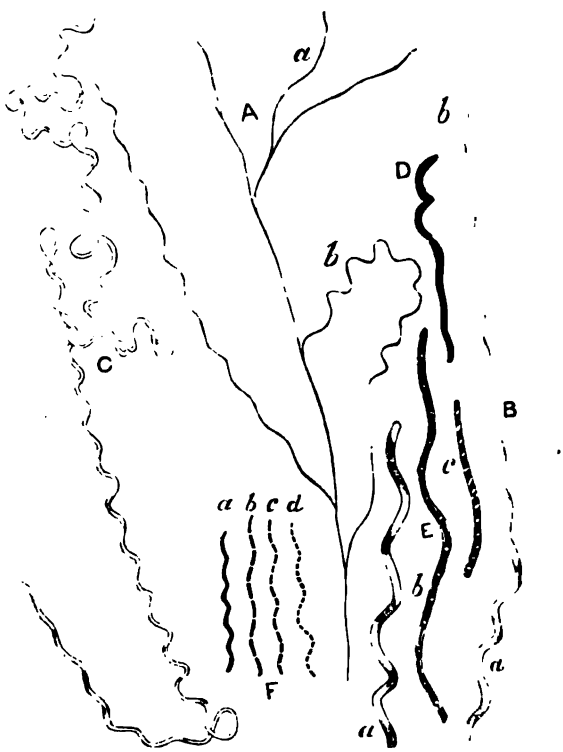


FIG. 66.

CLADOTHRIX DICHOTOMA—A. Branched Schizomycete: (*a*) Vibrio-form; (*b*) Spirillum-form [slightly magnified]. B. Screw-form at the ends: (*a*) Spirillum-form; (*b*) Vibrio-form. C. Very long Spirochæta-form. D. Branch fragment, at one end Spirillum-form, at the other Vibrio-form. E. Screw-form: (*a*) Continuous; (*b*) Composed of rods, and (*c*) Cocci. F. Spirochæta-form: (*a*) Continuous; (*b*) Composed of long rods, (*c*) Short rods, and (*d*) Cocci [after Zopf].

duction is not sufficiently known to afford a better means for distinction than the other morphological appearances taken alone; nor can we depend upon physiological action, which is held by many to vary with the change of form, according to altered surroundings.

Zopf, who has warmly supported the pleomorphism of bacteria, has suggested as a result of his investigations a division of the *Schizomycetes*, *Spaltpilze*, or *Fission-fungi*, into the following four groups:—*

1. **Coccaceæ.**—Possessing (so far as our knowledge at present reaches) only cocci, and thread forms resulting from the juxtaposition of cocci. The fission occurs in one or more directions.

Genera:—Streptococcus, Micrococcus, Merismopedia, Sarcina, Ascococcus.

2. **Bacteriaceæ.**—Possessing mostly cocci, rods (straight or bent), and thread-forms (straight or spiral). The first may be absent, and the last possess no distinction between base and apex.

Division (as far as is known) occurs only in one direction.

Genera:—Bacterium, Spirillum, Vibrio, Leuconostoc, Bacillus, Clostridium.

3. **Leptotricheæ.**—Possessing cocci, rods, and thread-forms (which show a distinction between base and apex). The last straight or spiral.

* Zopf, *Die Spaltpilze*, 1885.

Genera :—Leptothrix, Beggiatoa, Crenothrix, Phragmidiothrix.

4. Cladotricheæ.—Possessing cocci, rods, threads, and spirals. Thread-forms provided with false branchings.

Genus :—Cladothrix.

Zopf, however, does not assert that all the fission-fungi exhibit this pleomorphism, nor does he pretend that his classification will include all the micro-organisms described. Cohn, on the other hand, was ready to admit that all the forms described by him were not truly independent species. Quite recently De Bary, Hueppe, Baumgarten, and Flügge have expressed new views with regard to the classification of bacteria.

De Bary divides them into two great groups—bacteria which form endospores, and bacteria which form arthrospores. This affords but little practical assistance, though regarded by botanists, from a scientific standpoint, as a step in the right direction.

Hueppe, acknowledging that the fructification must eventually be made the basis for classification, suggests an arrangement for provisional use in which this view is introduced.

HUEPPE'S CLASSIFICATION.

- **Cocci forms**
  - Arranged in chains
    - Zooglœa moderate — Spores endogenous (?) — *Streptococcus (?).*
    - Zooglœa strongly marked — Arthrospores or spores unknown — *Arthro-streptococcus*
    - *Leuconostoc.*
  - Arranged in fours
    - With small chains — *Merista.*
    - Without small chains — *Sarcina.*
  - Arranged in eights
  - In irregular heaps
    - Zooglœa indeterminate — *Micrococcus.*
    - Zooglœa united in balls — *Ascococcus.*
- **Rod forms**
  - Smaller or longer threads, without distinction of base and apex. Threads flexible or rigid
    - Threads straight or wavy, no endogenous spores — *Artho-bacterium.*
    - Threads straight, wavy, or screw form, no endogenous spores — *Spirulina.*
    - Threads straight or wavy, spores endogenous
      - Without change of the cells — *Bacillus.*
      - With change of the cells, spindle rods — *Clostridium.*
  - Threads with distinction of base and apex
    - Threads without divisions
      - Without presence of sulphur — *Leptothrix.*
      - With presence of sulphur — *Beggiatoa.*
    - Threads with divisions
      - Unbranched — *Crenothrix.*
      - Branched — *Cladothrix.*
- **Screw rods**
  - Screw-like threads, flexible or rigid
    - Formation of arthrospores or fructification unknown — *Spirochæta.*
    - Endogenous spores
      - With change of form — *Vibrio.*
      - Without change of form — *Spirillum.*

It has already been mentioned that the production of arthrospores is only established in a very few species. Therefore, we are hardly justified in assuming that all bacteria, the spore-formation of which is quite unknown, are to be included with those in which this kind of fructification has been observed, and consequently to distinguish genera on the same grounds may be considered, to say the least, somewhat premature. In Baumgarten's classification the genus bacterium is dispensed with, and the genera divided into two groups, the monomorphic and the pleomorphic.

GROUP I.—MONOMORPHIC.
Genera.—Coccus.
Bacillus.
Spirillum.

GROUP II.—PLEOMORPHIC.
Genera.—Spirulina.
Leptothrix.
Cladothrix.

Flügge also, in his recent classification, includes the genus bacterium in the genus bacillus. The new classification differs also from the original one in the grouping together of the different species according to the character and behaviour of the colonies in nutrient gelatine. The abolition, in Flügge's and Baumgarten's classification, of the genus bacterium is no doubt owing to confusion having arisen from the distinction between a

bacterium and a bacillus, being a question of length. Observers differed as to whether a rod of a certain length ought to be considered a bacterium or a bacillus. To meet this difficulty a rough-and-ready rule was suggested, viz., that a rod less than twice its breadth in length should be considered as a bacterium, and otherwise a bacillus. But this purely arbitrary division was inadequate, from the fact that a rod at one stage of its growth or under certain

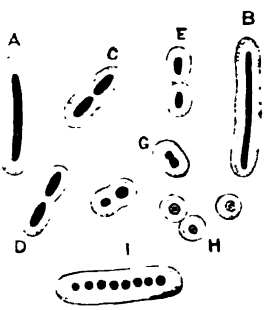


FIG. 67.—BACTERIUM PNEUMONIÆ CROUPOSÆ, × 1500 (after Zopf).

conditions might, as far as length went, truly be a bacterium, and under other circumstances be of such a length as to entitle its being considered a bacillus. We avoid such confusion if we follow Zopf, and acknowledge as a difference between a bacterium and a bacillus the presence or absence of that form of spore-formation now distinguished as endogenous spore-formation. We can then most conveniently retain this generic term, to include that group of rod-forms in which this spore-formation is as yet unknown; moreover, we shall find that by so

doing, with one or two exceptions, we get collected together those short rod-forms (Fig. 67), which appear to link the simple cocci to the spore-bearing rods or bacilli.

This must surely lead to less confusion than

FIG. 68.—EMMERICH'S BACTERIUM, × 700 (after Emmerich).

regarding all rod-forms as bacilli, and massing them together into one genus. For by those who adopt the latter plan, not only are very short rods with rounded ends included as bacilli, *e.g.*, *Bacillus*

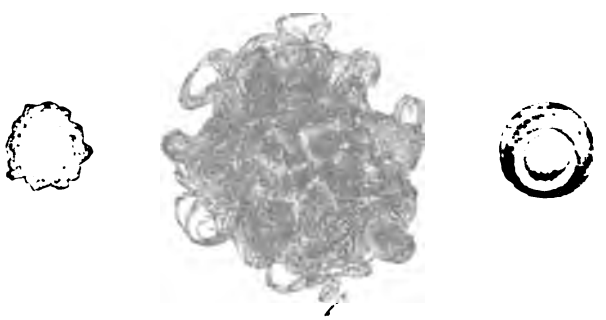


FIG. 69.—COLONIES ON NUTRIENT GELATINE, × 60.

Neapolitanus or Emmerich's bacterium (Fig. 68), but even cells which are described as ovoid are also regarded as bacilli, as in Löffler's so-called *Bacillus parvus ovatus*.

The grouping together of the different species according to the character of the colonies in nutrient gelatine (Figs. 69, 70) is also of questionable advisability. These characters can hardly

FIG. 70.—COLONIES ON NUTRIENT AGAR-AGAR, × 60.

be considered to be of sufficient importance, or indeed in many cases to be sufficiently constant, to serve by themselves for this purpose. In many cases a slight variation in the composition of the

FIG. 71.—COLONY OF BACILLUS ANTHRACIS, × 60. From a cover-glass impression-preparation, stained with gentian-violet.

nutrient medium may considerably affect the appearances of the colonies. At the same time, the appearances are very characteristic of certain species of bacteria (Fig. 71), and in other

cases the characters of the colonies, together with the characters of the growths in test-tubes, assist us in distinguishing species which are morphologically similar, as in the case of the comma bacilli of Finkler and of Koch.

The classification here given will be found to be a convenient form for arranging the micro-organisms for reference, and it may lead the investigator to work upon the same lines as Zopf, and by tracing the life-history of individual forms in pure cultivations, either to extend the work of establishing protean species or to restrict the

FIG. 72.—BACTERIUM OF RABBIT SEPTICÆMIA.

doctrine of pleomorphism to a very few forms. But though the author adheres to the lines of classification proposed by Zopf, he is not prepared to accept his teachings in their entirety; thus, to embrace all described species, and to be consistent with the author's views, it has been necessary, not only to add to Zopf's classification, but in many cases to modify his arrangement of species. For instance, Zopf regards the bacterium of rabbit septicæmia (Fig. 72) as a micrococcus, and the yellow coccus in pus, which may occur in short chains, as a micrococcus. Of some species alteration in the nomenclature is justified by necessity.

Thus the well-known *Micrococcus prodigiosus*, now named by Flügge and others as *Bacillus prodigiosus*, is placed by the author as a bacterium, for it undoubtedly appears in the form of short rods, but without endogenous spore-formation.

Any arrangement at present can only be considered provisional, and, therefore, that arrangement which is of most practical assistance, and which leads to a clear description of the important characteristics on which the final classification will depend, must be considered to be the best. For example, if we abolish the genus bacterium, any rod-form may be at once classed as a bacillus; on the other hand, in the plan here adopted, we must determine the presence or absence of endogenous spore-formation before we can decide whether it be a bacterium or a bacillus. This necessarily leads to a more thorough study of their life-history. In the systematic description which follows, stress is laid upon the morphological appearances of bacteria, upon the absence or presence of spore-formation, and upon the appearances under cultivation, in addition to other characteristics, such as the changes produced by their growth. The determination of species rests upon the accumulated evidence afforded by a thorough knowledge of their life-history. The form of the organism, the changes it effects, and the microscopical appearances under cultivation, must be collectively taken into account.

CHAPTER XII.

SYSTEMATIC AND DESCRIPTIVE.

THE *Schizomycetes*, *Spaltpilze*, or *Fission-fungi* have already been described as divisible, according to Zopf, into four groups; *Coccaceæ*, *Bacteriaceæ*, *Leptotrichieæ*, and *Cladotrichieæ*. They comprise the following genera and species :—

GROUP I.—COCCACEÆ.

Genus I. *Streptococcus* (Chain-cocci).—Division in one direction only. Individual cocci always or occasionally remain united together to form chains.

Genus II. *Merismopedia* (Plate-cocci).—Division in two directions, forming lamellæ or plates.

Genus III. *Sarcina* (Packet-cocci).—Division in three directions, forming colonies in cubes or packets.

Genus IV. *Micrococcus* (Mass-cocci).—Division in one direction, cocci after division may remain aggregated in irregular heaps, but never form chains.

Genus V. *Ascococcus* (Pellicle-cocci).—Like micrococcus, but the cocci grow in characteristic gelatinous pellicles.

Genus I.—Streptococcus.

SPECIES.

ASSOCIATED WITH DISEASE (*many Pathogenic or causally related to disease*) :—

In man	Streptococcus pyogenes	Pathogenic in man and animals.
	Streptococcus pyogenes malignus	Saprophytic in man, pathogenic in animals.
	Streptococcus pyogenes aureus	Pathogenic in man and animals.
	Streptococcus pyogenes albus	,, ,,
	Streptococcus pyogenes citreus	,, ,,
	Streptococcus cereus albus	Associated with pus, but not pathogenic in man or animals.
	Streptococcus cereus flavus	,, ,,
	Streptococcus erysipelatosus	Pathogenic in man and animals.
	Streptococcus toxicatus	Pathogenic (?).
	Streptococcus in puerperal fever	Possibly saprophytic only.
	Streptococcus in endocarditis	,, ,, ,,
	Streptococcus in diphtheria	,, ,, ,,
	Streptococcus articulorum	Saprophytic in man, pathogenic in animals.
	Streptococcus in cerebro-spinal meningitis	Possibly saprophytic only.
	Streptococcus in yellow fever	,, ,, ,,
	Streptococcus in dental caries	,, ,, ,,
	Streptococcus variolæ et vacciniæ	Pathogenic in man (?) and animals (?).
	Streptococcus of swine-erysipelas	Pathogenic (?).
	Streptococcus of cattle-plague	Pathogenic.
In animals	Streptococcus of foot and mouth disease	,,
	Streptococcus of septicæmia consecutive to anthrax	,,
	Streptococcus septicus	,,
	Streptococcus of progressive tissue necrosis in mice	,,
	Streptococcus perniciosus	Pathogenic (?).
	Streptococcus bombycis	Pathogenic.
	Streptococcus insectorum	Possibly saprophytic only.

UNASSOCIATED WITH DISEASE :—

Streptococcus viscosus	Zymogenic saprophyte.
Streptococcus coronatus	Simple saprophyte.
Streptococcus radiatus	,, ,,
Streptococcus flavus desidens	,, ,,

Streptococcus pyogenes, Rosenbach (*Chain micrococci in pus: Coccus of Pyæmia*). — Cocci occurring singly, in chains, and in zooglœa. Inocu-

lation of guinea-pigs or mice with pus containing these cocci, or with a pure cultivation of the same, causes suppuration at the site of injection, and death with symptoms of blood-poisoning. In the blood, in the tissue around the abscess, and in the pus the cocci are found singly or in zoogloea or chains. Their appearances in cultivation-media have been very minutely described.* Cultivated in a streak on the surface of nutrient gelatine on a glass plate, they form at first whitish, somewhat transparent, rounded spots, of the size of small grains of sand. They develop but slightly on this medium, even at the highest temperature attainable without liquefying the gelatine. On nutrient agar-agar they grow most energetically at a temperature of 35°—37° C. On this medium also, they show a tendency to form little spots, which finally become about the size of a pin's head. If a streak is made with a needle well charged with a fresh cultivation, growth in a continuous line is obtained, but still showing an inclination to form centres. In its further development the middle of the cultivation is heaped up, and presents a pale brownish coloration, while the periphery is flattened, except at the extreme margin, which is again raised up, and often with a spotted appearance. Still later, the periphery develops successive layers or terraces. The growth is so slow that

* Rosenbach, *Mikro-organismen bei den Wund-Infections-Krankheiten des Menschen.* 1884.

in two to three weeks the maximum width of the culture-streak is about 2 to 3 mm. On solidified blood serum the cocci grow as on agar-agar. They do not liquefy any nutrient medium. In a vacuum they rapidly cause the decomposition of white of egg or beef, which are energetically peptonised. They occur in acute abscesses.*

Streptococcus pyogenes malignus. Flügge. —Cocci occurring singly and in chains. They grow very slowly in nutrient gelatine; the cultivations closely resemble those of *Streptococcus pyogenes.* They are pathogenic in mice and rabbits. They were isolated from necrotic patches in the spleen of a fatal case of leukæmia.

Streptococcus pyogenes aureus† (*Staphylococcus** *pyogenes aureus*, Rosenbach. *Yellow coccus in pus. Coccus of acute infectious osteomyelitis*).—Cocci singly, in pairs, short chains, and irregular masses. Cultivated on nutrient agar-agar an orange-yellow culture develops, looking like a streak made with oil paint‡ (Plate VIII., Fig. 2). Cultivated in a test-tube of nutrient gelatine, the gelatine is rapidly liquefied, and the growth subsides as an orange-

* Ogston, *Brit. Med. Journ.* 1881.

† These cocci are placed among the Streptococci as they undoubtedly may occur in small chains of three or four individuals linked together. The form of zooglœa is not considered important. The same applies to the Staphylococcus cereus albus and flavus, which are also placed by the author in the genus Streptococcus. Passet acknowledges that they form long and short chains, but named them Staphylococcus because of the prevalence of irregular zooglœa.

‡ Rosenbach.

yellow sediment. On potatoes and blood serum a similar orange-yellow culture grows luxuriantly.

The micro-organisms injected into the pleura or knee of a rabbit produce, as a rule, a fatal result on the following day, but if it survives longer, it eventually dies of severe phlegmon. If injected into the knee of a dog, suppuration occurs, followed by disintegration of the joint. The cocci do not cause any septic odour in pus, nor does any gas develop. Albumen is converted by their action into peptones.

They occur in the pus of boils and in the abscesses of pyæmia, puerperal fever, and acute osteomyelitis. Injected into the peritoneal cavity of animals, they set up peritonitis, and introduced into the jugular vein they produce septicæmia and death. When a small quantity of a cultivation was introduced into the jugular vein after previous fracture or contusion of the bones of the leg, the animal died in about ten days, and abscesses were found in and around the bones, and in some cases in the lungs and kidneys. Similar cocci were found in the blood and pus of the animals.*

Streptococcus pyogenes albus (*Staphylococcus pyogenes albus*, Rosenbach). Cocci microscopically indistinguishable from the above. In cultivations also they resemble the *Streptococcus pyogenes aureus*, but the growth consists of opaque white masses. They liquefy nutrient gelatine

* Becker, *Deutsche Med. Wochenschr.* Nov. 1883.

rapidly, and subside to the bottom as a white sediment. They are also similar to the above-mentioned in their pathogenic action. Pure cultivations of the organism were obtained from a case of acute suppuration of the knee-joint.

Streptococcus pyogenes citreus (*Staphylococcus pyogenes citreus*,* Passet). Cocci singly, in pairs, chains, and irregular masses. If cultivated on nutrient gelatine or nutrient agar-agar, a sulphur or lemon-yellow growth develops (Plate XXIV., Fig. 3). When inoculated under the skin of mice, guinea-pigs, or rabbits, an abscess forms after a few days, from which a fresh cultivation of the micro-organism can be obtained. They are frequently present in pus.

Streptococcus cereus albus (*Staphylococcus cereus albus*, Passet).* Cocci, morphologically similar to the above, but distinguished by forming on nutrient gelatine a white, slightly shining layer, like drops of stearine or wax, with somewhat thickened, irregular edge. The needle track develops into a greyish-white, granular thread. In plate-cultivations, on the first day, white points are observed, which spread themselves out on the surface to spots of 1—2 mm. When cultivated on blood-serum a greyish-white, slightly shining streak develops, and on potatoes the cocci form a layer which is similarly coloured.

* Passet, *Fortschritte der Medicin*, Jan. 15th and Feb. 1st, 1885.

Streptococcus cereus flavus (*Staphylococcus cereus flavus*, Passet).*—Cocci which also occur in pus. If cultivated in nutrient jelly the growth, which is at first white, becomes lemon-yellow, somewhat darker in colour than *Streptococcus pyogenes citreus*. Microscopically *Streptococcus cereus flavus* corresponds with *Streptococcus cereus albus*, and they both form zooglœa of medium-sized cocci (diam. 1·16 μ). Inoculation experiments with both kinds gave negative results. Among the micro-organisms present in pus a coccus has been described as occurring occasionally, which is almost identical with *Bacterium pneumoniæ crouposæ;* compare also *Streptococcus pyogenes* (p. 195) and *Streptococcus pyogenes aureus* (p. 197).

Streptococcus erysipelatosus.—Minute cocci ·4 μ to ·3 μ occur in chains in human erysipelatous skin, and in the fluid of erysipelatous bullæ. They occupy the lymphatic channels of the skin, and spread along them as the disease progresses.†

They can be cultivated artificially in nutrient gelatine or agar-agar, and produce typical erysipelas when re-inoculated in man or animals.‡ The characteristic erysipelatous blush is produced by inoculating these micro-organisms in the ear of a rabbit. In the human subject the disease was produced in fifteen to sixty hours after inoculation.

* Passet, *ibid.*

† Lukomsky, *Virch. Archiv*, vol. lx.

‡ Orth, *Archiv für Exp. Pathol. u. Pharmacol.*, Bd. i. 1873.

A beneficial result was obtained in cases of lupus, cancer, and sarcoma, this being the object for which the latter inoculations were undertaken.*

The appearances of cultivations very strongly resemble those already described in *Streptococcus pyogenes*. There is less tendency, however, to the formation of terraces, the edge of the growth is thicker and more irregular, and the appearance of the streak is more opaque and whiter.†

Streptococcus toxicatus, Burrill.—Globular cells, ·5 μ in diam., singly, in pairs, and rarely in chains. They occur in species of *Rhus* (*Sumach*), and have been credited with being the cause of the inflammation which is produced by the poison of the plant.

Streptococcus in puerperal fever.‡—Cocci in zooglœa, and sometimes in chains, have been observed in all organs affected in puerperal fever, and especially in the endocardium, lung, spleen, kidney, and brain.

Streptococcus in endocarditis, Klebs.—Cocci 1 μ and ·5 μ in diam., and chains. They have been observed in masses upon the altered valves and in the detritus of the ulcerations of the endocardium

* Fehleisen, *Aetiologie des Erysipels*. 1883.

† Rosenbach.

‡ In this and many similar cases the cocci are given as distinct species from their association with particular diseases, not because they are believed to be causally related, for there is very little evidence in favour of that belief as yet, but purely for convenience of reference. In many cases they are probably only septic organisms, which have found a pabulum in the dead tissue; others appear to be identical with organisms which have been found in pus.

in *endocarditis ulcerosa;* as chains also in the muscle of the heart, and forming plugs in the vessels of the heart, spleen, and kidney. Some forms are identical with *Staphylococcus pyogenes aureus.** Micrococci have also been described in connection with chronic and other forms of endocarditis.

Streptococcus in diphtheria.—Oval cocci, ·35 to 1·1 μ in diameter, have been described as characteristic of diphtheria.† The cells lie singly, in pairs or in rosaries, and in spherical or cylindrical masses in diphtheritic membranes and the surrounding connective and muscular tissues. From the point of infection they can be traced along the lymphatics, and are found in the blood, heart, liver, kidneys, and other organs. In severe cases the blood capillaries and uriniferous tubules are blocked up.

The attempt to get pure cultivations in nutrient media and to make inoculation experiments has not yet succeeded; a bacterium has also been described as associated with diphtheria (p. 230).

Streptococcus articulorum, Löffler.—Cocci forming remarkably long chains. Cultivated in nutrient gelatine they form pale-grey watery-looking droplets. They frequently produce a fatal result when inoculated in mice, and the chains are found in the spleen and other organs. Rabbits inoculated subcutaneously in the ear

* Ziegler, *Patholog. Anatomy.* 1885.
† Oertel, *Deutches Archiv f. Klin. Med.* 1871.

suffered from an erysipelatous inflammation. Injection of the cocci into the circulation produced suppurative inflammation of the joints. Isolated from the mucous membrane of diphtheritic cases.

Streptococcus in cerebro-spinal meningitis.* —Cocci, diplococci, and chains have been observed in the exudation of cerebro-spinal meningitis; all forms were detected in the meninges, and zooglœa in the kidneys.

Streptococcus in yellow fever.—Cocci ·6-·7 μ in diameter have been observed in this disease.† They occur in chains, aggregated in masses, which distend the vessels of the kidney and liver.

Streptococcus in dental caries.—Two species of streptococci are believed to be intimately connected with caries of the teeth.‡

1. Occurs in the form of cocci, diplococci, and chains, which develop very rapidly in nutrient gelatine, speedily converting it into a turbid liquid. They are agents of lactic acid fermentation.

2. Occurs as very small cocci, rarely in chains, which rapidly liquefy nutrient gelatine. There are also associated with these, two species of micrococci and a spirillum (*Spirillum sputigenum*).

Streptococcus variolæ et vacciniæ.—Cocci, ·5 μ in diam., singly, in pairs, and in long or short chains, and colonies. They are found in the fresh

* Leyden, *Central Blatt f. Klin. Med.* 1883.
† Cornil and Babes, *Les Bactéries.* 1885.
‡ Miller, *Deutsche Med. Woch.* 1884.

lymph * of human and cow-pox, and in the pustules of true small-pox. They are regarded as the active principle of vaccine lymph, since filtration deprives the latter of its infectious element.† The lymphatics of the skin in the region of the pustule of both human and sheep-pox are filled with cocci. Successful vaccination has been stated to result from artificial cultivations.‡

Streptococcus of swine-erysipelas (*Microbe du rouget du porc*, Pasteur).—Cocci,§ diplococci, chains, and zooglœa have been described as present in the blood in "*rouget du porc.*" The microbes have no effect upon fowls, but kill rabbits and sheep. Inoculated into healthy pigs they give rise to the disease, and occasion a fatal result. Inoculation with weakened virus protects against virulent matter.

Streptococcus of cattle-plague, Semmer.—Cocci occurring singly in chains and zooglœa. They grow rapidly in artificial media. A calf inoculated from a cultivation died in seven days from cattle-plague. The cocci lose their virulence by successive cultivation, and the weakened cultivations protect against the virulent disease. They were observed in the blood and lymphatic glands, and cultivated from the latter.

* Cohn, *Virchow's Archiv.* 1872.

† Chauveau, *Comptes Rendus.* 1868. Burdon Sanderson, Reports on the intimate pathology of contagion.

‡ Quist, *St. Petersburgh Med. Wochenschrift.* 1883.

§ According to Löffler these cocci are associated with a minute bacillus which is the true cause of the disease (*vide* Bacillus of swine-erysipelas).

Streptococcus of foot and mouth disease, (*Micrococcus of foot and mouth disease*, Klein).—Cocci singly, in dumb-bells, and in curved chains. They grow well in milk, in alkaline peptone-broth, in nutrient gelatine, and in nutrient agar-agar. Cultivated on obliquely solidified nutrient gelatine they form a film composed of minute granules or droplets closely placed side by side, but not confluent. The gelatine is not liquefied. Observed in the vesicles of sheep suffering from foot and mouth disease.

Streptococcus of septicæmia consecutive to anthrax, Charvin.—In rabbits, some hours after death from anthrax, cocci in chains have been found in the kidney and elsewhere. Inoculated into rabbits fatal septicæmia was produced without the presence of suppuration.

Streptococcus septicus, Nicolaier.—Cocci, diplococci, and chains. They grow very slowly in nutrient media, forming in plate-cultivations after several days minute dot-like colonies. In mice they produce paralysis of the hind extremities and death, and in rabbits a local inflammation and death after two or three days. Isolated from earth.

Streptococcus of Progressive Tissue Necrosis in Mice.*—Cocci ·5 μ in diam., in chains or rosaries, and zooglœa; their invasion causes tissue necrosis with destruction even of cartilage

* Koch, *Untersuch. über die Ætiologied. Wundinfections Krankheiten.* 1878. [New Syd. Soc.]

cells, and spreading from the point of inoculation, causes death in about three days (Fig. 73). The cocci are absent from the blood and internal organs. These observations were made after the injection of putrid fluids in the ear of mice, and a pure infection was obtained by the inoculation of field-mice, which have an immunity from bacillary septicæmia.

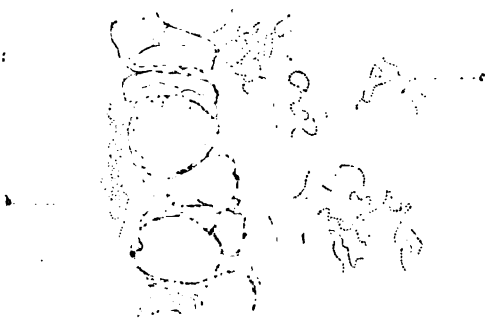


FIG. 73.—STREPTOCOCCUS OF PROGRESSIVE TISSUE NECROSIS IN MICE. (*a*) Necrotic cartilage cells, and (*b*) Chains in masses; (*c*) Chains isolated [after Koch].

Streptococcus perniciosus (*Parrot disease*). —Cocci, singly, in chains, and in zooglœa have been described in connection with a disease of the grey parrot (Psittacus erithacus).* This disease is fatal to about 80 per cent. of these parrots imported to Europe. They suffer from diarrhœa and general weakness; their feathers are ruffled, their wings hang loosely, and their eyelids close; convulsions set in, and death follows. At the

* Wolff, *Virchow's Archiv.* 1883.

autopsy greyish nodules are found in the lungs, liver, spleen, and kidney; in and around the capillaries of these nodules, and in the blood of the heart, the cocci are found in great numbers in zooglœa, and more rarely in chains. Inflammatory change in the surrounding tissue is absent.

Streptococcus bombycis, Béchamp (*Microzyma bombycis*).—Oval cocci ·5 μ in diam., singly, in pairs, and chains. They occur in the contents of the alimentary canal, and in the gastric juice of silkworms suffering from "*flacherie*" ("*Maladie de morts blancs*," "*flaccidezza*," "*schlafsucht*").

Streptococcus insectorum, Burrill.—Obtusely oval cells, ·7—1 μ long and ·55 μ broad, singly, in pairs, chains, or zooglœa. They were detected in the digestive organs of the chinck-bug (*Blissus leucopterus*) when suffering from a certain contagious disease.

Streptococcus viscosus, Pasteur.—Globular cells ·2 μ in diam., singly or in chains. These and allied forms have been considered to be the cause of mucoid fermentation in wine and beer* (*vin filant, bière malade*).

Streptococcus coronatus (*Micrococcus coronatus*, Flügge).—Cocci 1 μ in diam., singly, in short chains, and in zooglœa. In plate-cultivations the colonies have a characteristic halo, formed by the liquefaction of the gelatine around the colony. Isolated from the air.

* Pasteur, *Etudes sur le Vin; sur la Bière.* 1866; 1876.

Streptococcus radiatus (*Micrococcus radiatus*, Flügge).—Cocci less than 1 μ in diam., singly, and in short chains. They rapidly form whitish colonies with a yellowish-green sheen. They liquefy the gelatine, the colonies sinking down, and after one or two days developing a circlet of rays. A peculiar ray-like appearance is characteristic also of the growth in test-tubes. Isolated from contaminated plate-cultivations.

Streptococcus flavus desidens (*Micrococcus flavus desidens*, Flügge).—Cocci, diplococci, and short chains. They form yellowish-white colonies, which gradually sink down in the gelatine. In test-tubes they form china-white, confluent masses in the track of the needle, and on the surface a yellowish-brown slimy layer. Isolated from contaminated plate-cultivations.

Genus II.—Merismopœdia.

SPECIES.

ASSOCIATED WITH DISEASE:—	
Merismopedia gonorrhœæ . .	Pathogenic in man.
Micrococcus tetragonus . .	Saprophytic in man. Pathogenic in animals.
Diplococcus albicans tardissimus	Saprophytic in man.
UNASSOCIATED WITH DISEASE:—	
Micrococcus citreus conglomeratus	Simple Saprophyte.
Micrococcus subflavus. . .	,, ,,
Micrococcus albicans amplus .	,, ,,

Merismopedia Gonorrhœæ (*Coccus of Gonorrhœa*).—Cocci 0·83 μ in diam., singly, in pairs, in tetrads, and zooglœa groups. They are

found in gonorrhœal pus adhering to the pus corpuscles and epithelial scales. Artificial cultivations have been carried out,* and the pathogenic character of the cocci established by inoculation.

Micrococcus tetragonus.—Cocci about 1 μ in diam., in groups of four (tetrads), surrounded by a hyaline capsule. They are found in the sputa of phthisical patients and in the walls of tubercular cavities. In a test-tube of nutrient gelatine they form an irregular white growth, more especially in the upper part of the needle track (Plate IV., Fig. 1). On the sloping surface of nutrient agar-agar thick, whitish, heaped-up masses develop. Guinea-pigs and mice inoculated with a minute quantity of a pure cultivation die in two to ten days, and the groups of the characteristic tetrads may be found in the capillaries throughout the body, especially in the spleen, lung, and kidney (Plate XII., Fig. 1).

Double infection can be produced by inoculating a mouse with a pure cultivation of *Bacillus anthracis* two or three days after inoculation with *Micrococcus tetragonus*. On examination after death the capillaries of the lungs, liver, and kidney are filled with both anthrax bacilli and masses of tetrads† (Plate XVII., Fig. 2).

Micrococcus citreus conglomeratus, Bumm.

* Bockhart, *Sitzungsberichte der Phys. Med. Gesell. Würzburg.* 1882.

† The Author, "Notes from a Bacteriolog. Laboratory," *Lancet*, 1885.

—Cocci 1·5 μ in diam., similar to gonococci. They form lemon-yellow colonies on plate-cultivations. Isolated from blennorrhœic pus and from dust from the air.

Diplococcus albicans tardissimus, Bumm.—Cocci morphologically identical with gonococci. They grow extraordinarily slowly on gelatine. They were isolated from urethral pus.

Micrococcus subflavus, Bumm.—Cocci morphologically resembling gonococci. Cultivated on nutrient gelatine, they form whitish dots which become gradually greyish and then yellow in colour, and confluent. They were observed in lochial discharges and vaginal secretions.

Micrococcus albicans amplus, Bumm.—Cocci in pairs and tetrads similar to gonococci, but considerably larger. Found in vaginal secretions.

Genus III.—Sarcina.

SPECIES.

UNASSOCIATED WITH DISEASE:—

Sarcina lutea Sarcina aurantiaca	Chromogenic saprophytes.
Sarcina ventriculi Sarcina intestinalis Sarcina urinæ Sarcina litoralis Sarcina Reitenbachii Sarcina hyalina Sarcina alba	Simple saprophytes.

Sarcina lutea.—Cocci singly, in pairs, in tetrads, and in packets. A single individual in a tetrad may be divided into two, or into four, so that a tetrad within a tetrad results. Cultivated

in nutrient agar-agar in a test-tube, they form a colourless growth along the track of the needle, and a bright canary-yellow layer upon the surface, where they have access to the air (Plate XIII., Fig. 1, Plate VIII., Fig. 1). In plate-cultivations the colonies are round, slightly granular in appearance, and yellow. Cultivated in a test-tube containing nutrient gelatine, they grow rapidly; the gelatine becoming liquid, the yellow growth forms a wad about the middle of the tube (Plate V., Fig. 2), or, liquefying the whole of the gelatine, subsides to the bottom of the test-tube. Cultivated on sterilised potatoes they form a yellow layer (Plate X., Fig 1). In drop-cultures in bouillon the subdivision into tetrads within tetrads and formation of groups of 8, 16, and 24 can be studied (Plate I., Fig. 7). Inoculation of mice produces negative results. The cocci are occasionally present in the air.

Sarcina aurantiaca.—Cocci singly, in pairs, in tetrads, and in packets. They form small orange-yellow colonies on plate-cultivations, and in test-tubes slowly liquefy the gelatine along the whole needle track, forming on the surface an orange-yellow growth. On potatoes they slowly develop the same pigment.

Sarcina ventriculi, Goodsir.*—Cocci reaching 4 μ in diam., united in groups of four, or multiples of four, producing cubes or packets with rounded-off corners. Contents of the cells are

* Goodsir, *Edinburgh Med. and Surg. Journal.* 1842.

greenish or yellowish-red. They occur in the stomach of man and animals in health and disease, and were first detected in vomit.

Sarcina intestinalis, Zopf.*—Cocci in groups of four or eight. Very regular in form; never in the large packets which occur in *Sarcina ventriculi*. They are found in the intestinal canal, especially the cæcum, of poultry, particularly fowls and turkeys.

Sarcina urinæ, Welcker.—Very small cocci, 1·2 μ in diam., united in families of 8 to 64. Observed in the bladder.

Sarcina litoralis, Oersted.—Cocci 1·2—2 μ in diam., bound together in 4 to 8 families, which, in their turn, may unite and include as many as 64 tetrads. Plasma colourless; in each cell 1—4 sulphur granules. Discovered in sea-water containing putrefying matter.

Sarcina Reitenbachii, Caspary.—Cocci about 1·5 to 2·5 μ in diam., at the time of division lengthened to 4 μ. Mostly united together from 4 to 8 in number; occasionally 16 or more. Colourless cell-wall, lined with rose-red layer of plasma. Found on rotting water plants.

Sarcina hyalina, Kützing.—Cocci round, 2·5 μ in diam., almost colourless. United in families of 4 to 24 cells, reaching 15 μ in diam. In marshes.

Sarcina alba.—Small cocci. They form small white colonies on nutrient gelatine. In test-tube cultivations they grow slightly along the needle

* Zopf, *Die Spaltpilze*. 1885.

track, but are heaped up on the surface without liquefying the gelatine. They are present in the air.

Genus IV.—Micrococcus.

SPECIES.

ASSOCIATED WITH DISEASE :—		
In man	Micrococcus in scarlatina	Possibly only saprophytic.
	Micrococcus in measles	
	Micrococcus in whooping cough	
	Micrococcus in hæmophilia neonatorum	
	Micrococcus in typhus	
	Micrococcus in acute yellow atrophy	
	Micrococcus in dental caries	
	Micrococcus in gangrene	
	Micrococcus pyogenes tenuis	
	Micrococcus in rabies	Pathogenic (?).
In animals	Micrococcus of septicæmia in rabbits	Pathogenic.
	Micrococcus of pyæmia in rabbits	
	Micrococcus of progressive suppuration in rabbits	
	Micrococcus parvus ovatus	
	Micrococcus of pyæmia in mice	
In plants	Micrococcus amylivorus	Pathogenic (?).
UNASSOCIATED WITH DISEASE :—		
	Micrococcus cyaneus	Chromogenic saprophytes.
	Micrococcus aurantiacus	
	Micrococcus chlorinus	
	Micrococcus violaceus	
	Micrococcus luteus	
	Micrococcus rosaceus	
	Micrococcus hæmatodes	
	Micrococcus candidus	Simple saprophytes.
	Micrococcus candicans	
	Micrococcus fœtidus	
	Micrococcus crepusculum	
	Micrococcus cinnabareus	
	Micrococcus flavus liquefaciens	
	Micrococcus flavus tardigradus	
	Micrococcus versicolor	
	Micrococcus viticulosus	
	Micrococcus lacteus faviformis	
	Micrococcus fulvus	

Micrococcus in scarlatina.—Cocci have been described in cases of scarlet fever as being present

in the blood,* in the scales of the desquamating epidermis,† and in the discharges and ulcerated tissue of the throat.

Micrococcus in measles.—Round cocci and diplococci have been observed in the catarrhal exudations, in the papules and in the capillary vessels of the skin, and in the blood of patients attacked with measles.‡

Micrococcus in whooping cough.—Elliptical cocci are said to be constantly present in the expectoration of persons suffering from whooping cough.§

Micrococcus in hæmophilia neonatorum, Klebs.—A coccus, which has been named *Monas hæmorrhagicum*, is stated to be characteristic of this disease.

Micrococcus in typhus.—Actively motile dumb-bell cocci have been described in the blood, and plugs of cocci in the lymphatics of the heart, in cases of typhus fever.‖

Micrococcus in acute yellow atrophy.—Cocci have been observed in the vessels of the liver in this disease.¶

Micrococcus in gangrene.—Oval and round cocci are found, which form zooglœa in the depth of gangrenous tissues. From gangrene of the lung

* Coze and Feltz, *Malad. Infect.* 1872.

† Pohl-Pincus, *Centralblatt f. d. Med. Wiss.* 1883.

‡ Keating, *Phil. Med. Times.* 1882. Cornil and Babes, *Les Bactéries.* 1885.

§ Bürger, *Berl. Klin. Woch.* 1883.

‖ Mott, *Brit. Med. Journal.* 1883.

¶ Eppinger, *Prager Vierteljahrsschrift.* 1875.

cocci have been isolated, which form greyish-white colonies in plate-cultivations of nutrient gelatine. In a test-tube of nutrient gelatine a growth results chiefly on the surface; the cultivations yield a penetrating odour.

Micrococcus pyogenes tenuis, Rosenbach.—Cocci, which, cultivated on agar-agar, form an opaque streak in the track of the needle with a transparent glassy growth extending from it. Occasionally found in the pus of closed abscesses.

Micrococcus in rabies.—Cocci have been described in connection with hydrophobia. The cocci were observed in sections of the spinal cord of rabid dogs. The descriptions given by different observers* vary considerably, and it is not yet ascertained whether any particular coccus is constantly associated with the disease. Nor have the organisms observed in stained preparations been cultivated apart from the diseased animal. By many, however, hydrophobia is believed to be due to the presence of a micro-organism, and researches which are being carried on in connection with attenuation of the virus still continue to excite the keenest interest† (p. 168).

Micrococcus of septicæmia in rabbits,

* Hermann Fol, *Académie des Sciences.* 1885. Babes, *Les Bactéries.* 1886. Dowdeswell, *Journ. Royal Microscop. Soc.* 1886.

† Pasteur, *Comptes Rendus.* 1882. *Congrès de Copenhague,* 1884. *Académie des Sciences.* 1885, 1886. Vignal, "Report on M. Pasteur's Researches on Rabies and the Treatment of Hydrophobia by Preventive Inoculation," *Brit. Med. Journal.* 1886.

Koch.*—Ellipsoidal cocci ·8—1 μ in largest diam. The disease was produced by the injection of putrid meat infusion. After death slight œdema was noted at the site of injection, slight extravasation of blood, and great enlargement of the spleen. No emboli or peritonitis resulted. Masses of cocci were found in the capillaries of different organs, especially in the glomeruli of the kidneys. Rabbits and mice inoculated with blood from the heart proved susceptible to the disease.

Micrococcus of pyæmia in rabbits, Koch.†—Round cocci and diplococci ·25 μ in diam. The disease was produced by the subcutaneous injection in a rabbit of distilled water, in which the skin of a mouse had been macerated. At the autopsy there were found great infiltration around the site of injection, peritonitis, and accumulations in the liver and lungs; in short, the appearances of pyæmia. In the capillaries of the organs examined, masses of cocci were observed enclosing blood corpuscles (Fig. 74). Fresh inoculations in rabbits with exudation-fluid, or blood from the heart, reproduced the same disease.

Micrococcus of progressive suppuration in rabbits, Koch.‡—Cocci only about ·15 μ in diam., principally in thick zooglœa. The disease was induced by the injection into rabbits of decomposing blood. At the place of injection a spread-

* Koch, *Wundinfect. Krankheit.* 1878.
† *Ibid.*
‡ *Ibid.*

ing abscess formed, which was fatal to the animal in about twelve days. No bacteria were observed in the blood, but in the walls of the abscess thick masses of cocci were found. The pus is infectious, causing the same disease in healthy rabbits.

Micrococcus parvus ovatus (*Bacillus parvus ovatus*, Löffler).—Small ovoid cocci, similar to the coccus of rabbit septicæmia. Cultivated on gelatine they develop readily a greyish-white growth at the

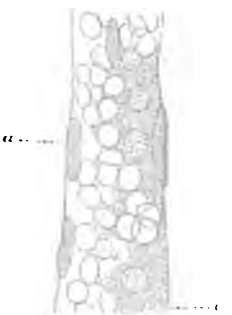


FIG. 74.—MICROCOCCUS OF PYÆMIA IN RABBITS; VESSEL FROM THE CORTEX OF THE KIDNEY, × 700. (*a*) Nuclei of the vascular wall; (*c*) Masses of micrococci adherent to the wall and enclosing blood corpuscles.

entrance of the inoculating needle. They are pathogenic in mice and rabbits. They proved also fatal in a pig after two days, producing œdema of the skin, inflammation of the mucous membrane of the stomach, but no effect on the general intestinal tract or mesentery. They were isolated from a pig suffering from a fatal disease simulating swine-erysipelas.

Micrococcus of pyæmia in mice, Klein.—Certain cocci which were present in pork broth

proved fatal to mice in about a week, producing purulent inflammation and abscess in the lungs. Fresh inoculations in mice again produced a fatal result with pyæmic symptoms.

Micrococcus amylivorus, Burrill.—Oval cells, 1—1·4 μ long, ·7 μ broad, singly, in pairs, and rarely in fours, never in chains, are found embedded in an abundant mucilage which is very soluble in water. They have been described as producing the so-called "fire blight" of the pear tree and other plants.

Micrococcus cyaneus, Cohn (*Bacteridium cyaneum*, Schröter).—Elliptical cells, growing upon cooked potato, and producing a blue colour. In nutrient solutions they form zooglœa, at first colourless, then bluish-green, and finally intense blue.

Micrococcus aurantiacus, Schröter.—Cocci, oval, 1·5 μ in diam., singly or in pairs, or in zooglœa. They occur as orange-yellow spots which coalesce into patches. A golden-yellow pellicle develops when they are cultivated in nutrient liquids. The colouring matter is soluble in water. They were observed on boiled potatoes and white of egg.

Micrococcus chlorinus, Cohn.—Cocci occur in the form of a finely granular zooglœa, causing a yellowish green or sap-green layer on boiled eggs and nourishing solutions. The colouring matter is soluble in water and is decolorised by acids.

Micrococcus violaceus, Schröter. — Cocci or elliptical cells, described as uniting into violet blue gelatinous spots, which again unite to form larger patches. They were observed on boiled potatoes exposed to the air.

Micrococcus luteus, Schröter.—Cocci similar in size to the above, elliptical, with highly refractive cell contents. They form yellow drops of 1—3 mm. diam. on boiled potato, and a thick, wrinkled, yellow skin on nutrient liquids. The colouring matter is insoluble in water, and unchanged by sulphuric acid or alkalies.

Micrococcus rosaceus.—Cocci forming pink colonies, and a rose-coloured growth on the surface of nutrient agar-agar. Observed contaminating an old cultivation (Plate XXIV., Fig. 2).

Micrococcus hæmatodes, Zopf. — Cocci, which, cultivated on boiled white of egg in a damp chamber in the incubator, form a red layer. The reaction of the colouring matter is similar to that produced by *Bacterium prodigiosum.* They have been observed in human sweat, especially from the axilla, colouring the surrounding parts and the linen an intense brick or blood-red colour.*

Micrococcus candidus, Cohn.—Cocci forming snow-white points and spots, upon slices of cooked potato. Possibly identical with the following :—

Micrococcus candicans, Flügge.—Cocci which collect in masses. In plate-cultivations they form

* Babes, "Vom Rothen Schweiss," *Biol. Centrabl.*, Bd. 2. 1882.

in two or three days milk-white colonies. Cultivated in test-tubes they form a white nail-shaped cultivation. They were isolated from contaminated plate-cultivations.

Micrococcus fœtidus, Rosenbach. — Small oval cocci. Cultivated in agar-agar they develop gas bubbles and a fœtid odour. Isolated from carious teeth. Possibly closely allied to, if not identical with, the following.

Micrococcus crepusculum, Cohn (*Monas crepusculum*, Ehrenberg. *Mikrokokken in fautenden Substraten*, Flügge). Round or short oval cells, scarcely 2 μ in diam.; singly or in zooglœa. They occur in various infusions and putrefying fluids in company with *Bacterium termo.*

Micrococcus cinnabareus, Flügge. — Large cocci occurring in twos, threes, and fours. In plate-cultivations they grow very slowly, forming punctiform colonies, in colour bright red at first, and afterwards reddish-brown. In test-tube cultivations they form on the surface of the gelatine a heaped-up red-coloured growth. Found contaminating old cultivations.

Micrococcus flavus liquefaciens, Flügge.— Cocci, diplococci, and zooglœa. On plate-cultivations they form yellowish colonies, and in test-tubes yellowish beads, which become confluent and rapidly liquefy the gelatine.

Micrococcus flavus tardigradus, Flügge.— Cocci forming chrome-yellow colonies. Cultivated

in test-tubes they form yellowish beads in the needle track, which remain isolated, and do not liquefy the gelatine. Isolated from contaminated cultures.

Micrococcus versicolor, Flügge.—Small cocci, which form iridescent colonies. In test-tubes they grow in the form of yellowish beads, and develop an iridescent layer on the surface.

Micrococcus viticulosus, Flügge. — Oval cocci, 1·2 μ in length, and 1 μ in width. In plate-cultivations the colonies differ when embedded in the nutrient medium and when growing on the surface. The characteristic appearance consists of a delicate network, which is visible also in test-tube cultivations in the track of the needle. On the surface of the medium they form a viscous layer. They were isolated from contaminated cultivations.

Micrococcus lacteus faviformis, Bumm and Bockart.—Cocci 1·25 μ in diam. Cultivated in gelatine they form milk-white confluent colonies, and preparations made from the cultivations have a characteristic honeycomb appearance. Isolated from vaginal secretions, and from sputum.

Micrococcus fulvus, Cohn. — Cocci round 1·5 μ in diam., frequently in pairs. They form rusty-red conical drops of a firm consistency, and about ·5 mm. diam., on horse dung.

Genus V.—Ascococcus.

SPECIES.

UNASSOCIATED WITH DISEASE :—
Ascococcus Billrothii . Zymogenic Saprophyte.

Ascococcus Billrothii.—Small globular cocci, united into characteristic colonies. They form on the surface of nourishing fluids a cream-like skin, divisible into an enormous number of globular or oval families. Each family is surrounded by a thick capsule of cartilaginous consistency. In a

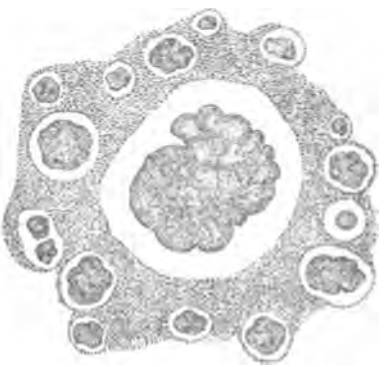


FIG. 75.—ASCOCOCCUS BILLROTHII [after Cohn].

solution containing acid tartrate of ammonia the fungi generate butyric acid, and change the originally acid fluid into an alkaline one. They were first observed on putrid broth, and later on ordinary nourishing solutions; they also readily develop upon damp slices of boiled roots, carrots, beetroots, etc.

METHODS OF STAINING COCCI.

Cocci stain well with watery solutions of gentian-violet, methyl-violet, fuchsine, methylene blue, and bismarck brown. For examining cocci in liquids such as pus or blood, or in cultivations in solid media, a little of the material should be spread out on a cover-glass (page 48), and stained with a drop or two of a watery solution of fuchsine or methyl-violet. The former is especially recommended for staining *Merismopedia gonorrhœæ.*

For a zoogloea, or pellicle of micrococci, Klein recommends transference bodily to a watch-glass containing the dye, leaving it there till deeply tinted, then taking it out with a needle, washing in water, and then in alcohol till excess of colour is removed. It must then be transferred to a glass-slide, spread well out, and a drop of clove-oil placed on it; after a minute or two the clove-oil is drained off, a drop of Canada balsam added, and covered with a cover-glass.*

Cocci in the tissues may be stained by immersing the sections in an aqueous solution of gentian-violet, or in aniline-gentian-violet solution, then rinsing in water, decolorising in alcohol, treating with clove-oil, and preserving in balsam (p. 58); or, after washing with alcohol, they may be rinsed with water, and stained for half an hour with Weigert's picrocarmine. From this they are again removed to water, then to alcohol, clove-oil, and Canada balsam.

The method of Gram is much more satisfactory (p. 59, Plate XII., Fig. 2). Sections should be examined with and without a contrast stain. The *after-stain* most commonly employed is eosin. The sections after the process of decolorisation should be placed in a weak alcoholic solution of eosin (two or three drops of a concentrated alcoholic solution added to a watch-glassful of alcohol), till stained a delicate pink. They are then rinsed in

* Klein, *Micro-organisms and Disease.* 1885.

fresh alcohol, treated with clove-oil, and preserved in Canada balsam.

Sections containing cocci of osteomyelitis may be *after-stained* with weak solution of vesuvin. Safranine and picro-lithium-carmine may also be used as contrast stains (p. 61).

Nuclear stains, such as carmine, hæmatoxylin, may also be employed. Sections may be left one minute in Grenacher's solution, then washed out in weakly acidulated alcohol (2—1000); and finally treated in the usual way, with alcohol, oil of cloves, and balsam.

Sections containing *micrococcus tetragonus* are best stained with Gram's method and eosin (Plate XII., Fig. 1), but they may also be treated by the method of Friedländer, to demonstrate their capsules (p. 227).

To stain the cocci of *rabbit-septicæmia* in the tissues, place the sections twenty-four hours in Löffler's solution, wash in water faintly acidulated with acetic acid, then treat with alcohol, oil of cloves, and balsam.

Group II.—Bacteriaceæ.

Genus I. *Bacterium.*—Cocci and rods, or only rods, which are joined together to form threads. Spore-formation absent or unknown.

Genus II. *Spirillum.*—Threads screw-form, made up of rods (long or short) only, or of rods and cocci. Spore-formation absent or unknown.

Genus III. *Leuconostoc.*—Cocci and rods. Spore-formation present in cocci.

Genus IV. *Bacillus.*—Cocci and rods, or rods only, forming straight or twisted threads. Spore-formation present either in rods or cocci.

Genus V. *Vibrio.*—Threads screw-form in long or short links. Spore-formation present.

Genus VI. *Clostridium.*—Same as bacillus, but spore-formation takes place in characteristically enlarged rods.

Genus I.—Bacterium.

SPECIES.

ASSOCIATED WITH DISEASE:—

In man	Bacterium pneumoniæ crouposæ	Pathogenic (?); possibly only saprophytic in man, pathogenic in animals.
	Bacterium pseudo-pneumonicum	Saprophytic in man, pathogenic in animals.
	Bacterium Neapolitanum	,, ,, ,,
	Bacterium in rhinoscleroma	Pathogenic in man (?).
	Bacterium in diphtheria	Saprophytic in man (?), pathogenic in animals.
	Bacterium saprogenes	Saprophytic in man, pathogenic in animals.
	Bacterium decalvans	Saprophytic.
In animals	Bacterium in diphtheria of calves	Pathogenic (?).
	Bacterium of diphtheria of pigeons	Pathogenic.
	Bacterium choleræ gallinarum	,,
	Bacterium septicum agrigenum	,,
	Bacterium of septicæmia in rabbits	,,
	Bacterium of Davaine's septicæmia	,,
	Bacterium septicum sputigenum	,,
	Bacterium crassum sputigenum	,,
	Bacterium pneumonicum agile	,,
	Bacterium oxytocum perniciosum	,,
	Bacterium cavicida	,,
	Bacterium coli commune	,,
	Bacterium lactis aërogenes	,,
	Panhistophyton ovatum	,,
In plants	Bacterium hyacinthi	Pathogenic (?).

UNASSOCIATED WITH DISEASE:—

Bacterium synxanthum	Chromogenic saprophytes.
Bacterium indicum	
Bacterium rubrum	
Bacterium prodigiosum	
Bacterium luteum	
Bacterium violaceum	
Bacterium brunneum	
Bacterium fluorescens putidum	
Bacterium fluorescens liquefaciens	
Bacterium ureæ	Zymogenic saprophytes.
Bacterium aceti	
Bacterium Pasteurianum	
Bacterium liodermos	Simple saprophytes.
Bacterium multipediculum	
Bacterium ramosum liquefaciens	
Bacterium Zopfii	
Bacterium merismopedioides	
Bacterium Pflügeri	
Bacterium photometricum	
Bacterium litoreum	
Bacterium fusiforme	
Bacterium navicula	
Proteus vulgaris	
Proteus mirabilis	
Proteus Zenkeri	
Bacterium termo	
Bacterium lineola	

Bacterium pneumoniæ crouposæ (*Pneumococcus*, Friedländer).—Cocci ellipsoidal and round, singly, or in pairs (diplococci), rods and thread forms. The cell-membrane thickens, and develops into a gelatinous capsule, which is round if the coccus is single, and ellipsoidal if the cocci occur in pairs or in rod-forms (Fig. 76, Plate I., Fig. 5). Cultivated in a test-tube of nutrient gelatine they grow along the needle track in the form of a round-headed nail (Plate IV., Fig. 2), without liquefaction

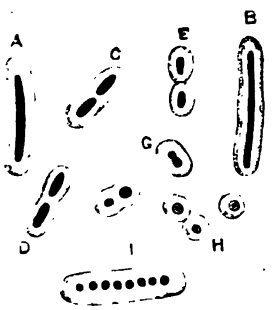


FIG. 76.—BACTERIUM PNEUMONIÆ CROUPOSÆ, FROM PLEURAL CAVITY OF A MOUSE, × 1500. A, B. Thread-forms. C, D, E. Short rod-forms. G. Diplococci. H. Cocci. I. Streptococci. [After Zopf.]

of the gelatine. The cocci when artificially cultivated have no capsule, but it again appears after their injection into animals. The cocci can also be cultivated on blood serum and on boiled potatoes. They occur in pneumonic exudation.* Inoculation of dogs with a cultivation of the cocci occasionally gave positive results; but in rabbits no results

* Friedländer, *Fortschr. d. Med.* 1883.

followed. Guinea-pigs proved to be susceptible in some cases, but thirty-two mice, after injection of a cultivation diffused in sterilised water into the lungs, died without exception. The lungs were red and solid, and contained the cocci, which were also present in the blood, and in enormous numbers in the pleural exudation. Inhalation experiments by spraying the cocci diffused in water into mouse cages succeeded in producing pneumonia and pleurisy in three out of ten mice. The nail-shaped cultivation is not always produced, nor are these conclusions accepted by all investigators.*

METHODS OF STAINING THE BACTERIA OF PNEUMONIA.

(*Pneumonie-Coccen*, Friedländer.)

Cover-glass-preparations (p. 48) of pneumonic sputum or exudation may be treated as follows:—

(*a*) Stain by the method of Gram, and after-stain with eosin (p. 59).

(*b*) Treat with acetic acid, then stain with gentian-violet or bismarck-brown. Examine in distilled water, or dry and preserve in Canada balsam.

(*c*) Float them on weak solutions of the aniline dyes twenty-four hours; differentiation between coccus and capsule is thus obtained.

(*d*) Stain with osmic acid; the contour of the capsules is brought out.

Sections of pneumonic lung should be stained by

(*a*) Method of Gram.

(*b*) *Method of Friedländer.* This method is employed to demonstrate the capsules in tissue sections. It consists

* Klein, *Micro-organisms and Disease.* 1885.

in placing the sections twenty-four hours in the following solution :—

Fuchsine	1
Distilled water	100
Alcohol	5
Glacial acetic acid . . .	2

They are then rinsed with alcohol, transferred for a couple of minutes to a 2 per cent. solution of acetic acid, and in the usual way treated with alcohol and oil of cloves, and preserved in Canada balsam.

Bacterium pseudo-pneumonicum (*Bacillus pseudo-pneumonicus*, Passet). Cocci round, oval, and occasionally elongated, similar to the bacterium of pneumonia. The oval forms are ·87 μ in width, and 1·16 μ in length. The colonies on plates appear in twenty-four hours as white dots; in test-tubes the growth develops as a greyish-white layer. If injected into the pleura they set up pleuritis, and into the abdomen peritonitis, in mice, rats, and guinea-pigs. Subcutaneous inoculation produces septicæmia in mice, and abscesses in rats, guinea-pigs, and rabbits. Inhalation experiments gave no results. They were isolated from pus.

Bacterium Neapolitanum (*Bacillus Neapolitanus*, Emmerich). Short rods with rounded ends. In width ·9 μ (Fig. 77). They form circular colonies, which later become irregular, granular, strongly refractive, and of a yellowish-brown colour. By introducing a large quantity into small animals changes were produced in the intestines with an

analogy to the post-mortem appearances of cholera. They are probably identical with bacteria found in healthy fæces. They were isolated from some cases of cholera at Naples.

FIG. 77.—BACTERIUM NEAPOLITANUM, × 700. (*a*) From intestinal contents in a case of cholera; (*b*) From peritoneal fluid of an inoculated guinea-pig [after Emmerich].

Bacterium of Rhinoscleroma (*Bacillus of Rhinoscleroma*, Cornil and Alvarez *). Cocci and short rods, 1·5—3 μ in length, ·5—·8 μ thick. Deeply coloured points or granules may occur in the course of the rods when stained, but it is very doubtful whether these can be considered as spores.

FIG. 78.—BACTERIA OF RHINOSCLEROMA. × 1400. Encapsuled cocci, diplococci, and short and long rod-forms [after Cornil].

The bacteria are encapsuled, the capsule being round when enclosing a coccus, and ovoid when enclosing a rod (Fig. 78). The capsule is composed of a tough resisting substance; two or more capsules may unite by fusion, enclosing two or three, or a great

* Cornil and Babes, *Les Bactéries*. 1885.

number of rods. The bacilli were observed in sections of a tumour, *rhinoscleroma*, which develops on the lip and on the nasal and pharyngo-laryngeal regions.

METHOD OF STAINING THE BACILLUS OF RHINOSCLEROMA.

Method of Cornil and Alvarez:—

Sections are immersed in a solution of methyl-violet (B) for twenty-four to forty-eight hours, with or without the addition of aniline-water; are then decolorised after treatment with the solution of iodine in iodide of potassium. If the sections are left to decolorise in alcohol for forty-eight hours the capsule is rendered visible.

Bacterium in diphtheria of man (*Bacillus of diphtheria*, Löffler).—Rods about the same length as the tubercle bacillus, but about twice as thick; the longer ones consist of single individuals linked together. Spores not observed. They were cultivated in a mixture consisting of three parts of calf's or lamb's blood serum, to which was added one part of neutralised veal broth, containing 1 per cent. peptone, 1 per cent. grape sugar, 1½ per cent. common salt. Cultivated in 5 per cent. gelatine at 20—22° C., the rods developed into irregular involution-forms. Inoculation gave doubtful results. The bacillus was isolated from diphtheritic membrane.* They were particularly noticeable in those typical cases

* Löffler, *Mittheil. a.d. K. Gesundheitsamte* (*Microparasites in Disease*, New Syd. Society).

characterised by a thick false membrane, extending over the fauces, larynx, and trachea. They occupied the deeper layers below the masses of bacteria which are found on the surface, such as the Streptococci already described (p. 202).

METHOD OF STAINING THE BACTERIA IN DIPHTHERIA.

Löffler's Method:—

Sections are placed in Löffler's solution for a few minutes, and excess of stain removed by ½ per cent. solution of acetic acid. They are then treated with alcohol and cedar-oil, and mounted in Canada balsam.

Bacterium saprogenes (*Bacillus saprogenes No.* 3, Rosenbach).—Rods isolated from the putrid marrow of a case of compound fracture. Cultivated on nutrient agar-agar, an ash-grey, almost liquid culture is developed, with a strong characteristic odour of putrefaction. Injected into the knee joint or abdomen of a rabbit, an opaque, yellowish-green infiltration resulted (*vide Bacillus saprogenes*, p. 314).

Bacterium decalvans, Thin.—Cocci, singly or in pairs, 1·6 μ in length. Observed in the roots of the hair in cases of *Alopecia areata*.

Bacterium in diphtheria of calves (*Bacillus vitulorum*, Löffler).—Rods about five or six times as long as wide, mostly united in long threads. A piece of tissue placed on blood serum developed a white layer composed of the bacilli. Successive generations were not obtainable. Mice inoculated directly from the calf died of a characteristic illness,

and the same long bacteria were again found in the inoculated animals accompanying widespread infiltration, starting from the point of inoculation. Inoculation of guinea-pigs and rabbits gave doubtful results. The bacteria were found in the deeper stratum of the diphtheritic patches.

Bacterium of diphtheria of pigeons (*Bacillus columbarum*, Löffler).—Short rods with rounded ends, mostly in irregular masses. In plate-cultivations on nutrient gelatine they formed whitish patches on the surface, and compact, ball-like masses when embedded in the gelatine. They were also cultivated on blood serum and potatoes. Subcutaneous inoculations in pigeons with a pure cultivation produced local inflammation and necrosis; inoculation in the mucous membrane of the mouth gave the appearances of the original disease. Other animals were only locally affected, except mice, in which characteristic symptoms and death resulted. They were isolated from the diphtheritic exudations in pigeons, and in sections were found in the vessels of the lungs and liver.

Bacterium choleræ gallinarum (*Micrococcus choleræ gallinarum*, Zopf. *Bacterium of Fowl-cholera. Microbe du choléra des poules*).—Cocci 2—3 μ in diam., short rods staining deeply at either pole, and longer beaded rods (Figs. 79, 80). In the tissues they appear mostly as rods 2 to 3 μ in length and 5 μ in diam., with their extremities stained more deeply than

their middle* (*vide* p. 135). When cultivated by introducing a drop of the infected blood into sterile chicken-broth, a number of round bodies, undergoing rapid movement and as a rule united as diplococci, or elongated and contracted in the middle, appear in the broth, which is at first slightly milky, but be-

FIG. 79.—BACTERIUM OF CHICKEN CHOLERA; BLOOD OF INOCULATED HEN, × 1200.

comes limpid, and the microbes at the same time pass into a finely granular state. From this, however, fresh cultures can still be started. Cultivated in a test-tube of nutrient gelatine, after from three

FIG. 80.—BACTERIUM OF CHICKEN CHOLERA, FROM MUSCLE JUICE OF INFECTED HEN, × 2500 [from a Photograph].

days to a week there develops along the needle track a fine, almost imperceptible, greyish thread without liquefaction of the gelatine (Plate III., Fig. 2). The growth is exceedingly scanty, even after several weeks.

Fowls suffering from the disease usually die very

* Cornil and Babes, *Les Bactéries*.

rapidly. In the less acute cases they are somnolent, weak in their legs, and their wings trail. They suffer from diarrhœa, and pass into a state of sopor and die. The micro-organisms are found in large numbers in the blood and organs after death, and in the intestinal discharges.

A drop of the broth injected into the connective tissue in the region of the pectoral muscles causes the death of the fowl the following day, with characteristic pathological changes.* If a culture be kept for some time, and a fowl be then inoculated with it, instead of death only local changes are produced, and the fowl is protected against the action of a virulent culture; thus affording an example of so-called *mitigation of the virus*.† The microbe is ærobic, and its toxic effect has been supposed to be due to the abstraction of oxygen from the blood producing asphyxia.

Bacterium septicum agrigenum (*Bacillus septicus agrigenus*, Nicolaier).—Cells morphologically similar to the microbe of chicken cholera. The colonies on plate-cultivations have a yellowish-brown centre, with a greyish-yellow zone. In test-tube cultivations the appearances are not characteristic. They are pathogenic in mice and in rabbits. The organs show no characteristic post-

* Cornil, "Observ. Hist. sur les Lésions des Muscles determinées par l'injection du Microbe du Choléra des Poules" (*Archives de Physiologie*. 1882); Cornil and Babes, *Les Bactéries*. 1885.

† Pasteur, "Sur le Choléra des Poules," *Compt. Rendus*. 1880.

mortem appearances, but the bacteria abound in the blood. They were isolated from earth.

Bacterium of septicæmia in rabbits (*Bacillus cuniculicida*, Koch).—Short rods, slightly pointed at both ends; in width ·6 μ—·7 μ, in length 1·4 μ. They stain deeply at the ends, leaving an uncoloured interval in the middle (Fig. 81),—an appearance which must be distinguished from a diplococcus or figure of eight. Two or more bacteria may be linked together in a chain. They may be cultivated in bouillon, blood serum, and nutrient gelatine. In plate-cultivations of the latter, they produce dot-

FIG. 81.—BACTERIUM OF RABBIT SEPTICÆMIA; BLOOD OF SPARROW, × 700 [after Koch].

like colonies, and in test-tubes little spherical masses in the needle track, and a layer on the free surface. The smallest quantity inoculated subcutaneously or in the cornea of a rabbit produces a rise of temperature and laboured breathing after 10—12 hours, and death in 16—20 hours. The spleen and lymphatic glands are found to be enlarged, and the lungs congested, but no extravasations, and no peritonitis. In the blood the characteristic rods abound, and in sections they are found in the vessels and capillaries. Mice and birds are very susceptible; guinea-pigs and white rats have

an immunity. The disease was produced by inoculating rabbits with contaminated water (River Panke) and with putrid meat infusion.

Bacterium of Davaine's septicæmia.—Rods similar to the bacteria described by Koch. They were also found in the blood of rabbits suffering from septicæmia, which, however, differed from Koch's septicæmia in that guinea-pigs were susceptible, and pigeons immune.

Bacterium septicum sputigenum (*Microbe de salive*, Pasteur. *Micrococcus Pasteuri*, Sternberg.* *Bacillus septicus sputigenus*, Fränkel).—Cocci oval, singly, in pairs, and in chains; often lanceolate or rod-shaped; encapsuled. They grow well in broth, and on agar-agar at 30° to 35° C. On the solid media they form a superficial, nearly transparent deposit of gelatinous consistence. They are pathogenic in rabbits, producing typical "sputum septicæmia." Fowls and dogs have an immunity, and guinea-pigs are less susceptible than rabbits. Mice die within forty-eight hours after being inoculated. The blood of an infected rabbit just dead is more potent than a liquid culture or than saliva containing the coccus. An animal which recovers after an injection of saliva is stated to be protected from the potent virus. The pathogenic power is modified by cultivation at a temperature between 39·5° and 40·5°. The organism has been supposed

* Sternberg, *Stud. Biol. Lab., John Hopkins, Univ.* II, No. 2, 1882. *Journal Royal Microscop. Society.* 1886.

to be intimately associated with croupous pneumonia, but any exact relation cannot be considered as established. The organism differs from the so-called *Bacterium pneumoniæ crouposæ* in that it is pathogenic in rabbits, it can be directly isolated from rusty sputum, and it requires a temperature for its growth at which nutrient gelatine is liquefied. The cocci were first observed in the blood of a rabbit inoculated with healthy saliva, and again found in a rabbit which died after inoculation with the saliva of a child suffering from rabies. Later they were isolated from the blood of rabbits inoculated with the buccal secretions of different individuals, and were found to be constantly present in the rusty sputum of pneumonic patients.

Bacterium crassum sputigenum, Kreibohm. —Short thick rods with rounded ends. Colonies on plate-cultivations appear as clear grey-white points, which ultimately form greyish slimy drops. In test-tubes they develop very quickly a nail-shaped growth. They are fatal to mice, and after death are found in the blood, and in sections, more especially in the capillaries of the liver. Rabbits die of septicæmia after intravenous injection. A large quantity of a cultivation injected into the circulation sets up fatal gastro-enteritis in rabbits and dogs in 3—10 hours. They were isolated from sputum

Bacterium pneumonicum agile (*Bacillus pneumonicus agilis*, Schou.).—Short thick rods, or

almost elliptical cells, often two to four linked together. They form dark granular colonies, which after twenty-four hours commence to liquefy the gelatine; a movement is then visible in the centre of the colony, and an appearance of circumferential rays results. They grow also on blood serum, bouillon, and potatoes. Cultures injected through the chest wall, or into the trachea, or administered by inhalation, set up pneumonia. They were isolated from pneumonic lungs of rabbit.

Bacterium oxytocum perniciosum (*Bacillus oxytocus perniciosus*, Wyssokowitsch).—Short rods with rounded ends, somewhat shorter and thicker than the bacterium of sour-milk. They form yellowish colonies, and in test-tubes develop a nail-shaped growth. Cultivated in milk they produced curdling, and an acid reaction. They were sometimes pathogenic in rabbits. Isolated from sour milk.

Bacterium cavicida (*Bacillus cavicida*, Brieger).—Very small rods, about twice as long as broad. They form colonies in the form of whitish concentric rings. On potatoes they develop dirty yellow tufts. They are very fatal to guinea-pigs. Isolated from human fæces.

Bacterium coli commune, Escherich.—Short, slightly-curved rods, 1·5 μ in length, ·3—·4 thick, colonies yellowish and granular. They develop a white scum on agar-agar and blood serum. Fatal to guinea-pigs and rabbits, when inoculated

intravenously. Isolated from fæces of infants fed exclusively on mothers' milk.

Bacterium lactis aerogenes, Escherich.—Short rods with rounded ends, 1·4—2 μ long, ·5 μ wide. Cultivations in gelatine resemble the bacterium of pneumonia. They produce fermentation in milk and in solution of grape-sugar. Pathogenic effects similar to the above. Isolated from the same source.

Panhistophyton ovatum, Lebert (*Nosema bombycis, Micrococcus ovatus, Corpuscles du ver à soie*).—Shining oval cocci, 2—3 μ long, 2 μ wide, singly and in pairs, or masses;* or rods, 2·5 μ thick, and twice as long.† They multiply by subdivision. They were experimentally proved to be the cause of *pébrine, gattine, maladie des corpuscles* or *Flecksucht;* and were discovered in the organs of diseased silkworms, as well as in the pupæ, moths, and eggs.

Bacterium hyacinthi, Wakker.—Cells resembling *Bacterium termo.* Observed in the yellow slime of diseased hyacinth bulbs.

Bacterium synxanthum, Ehrenberg (*Bacterium xanthinum. Bacterium of yellow milk*).—Cocci ·7—1 μ in length, and rod-forms.‡ They produce a yellow colour in boiled milk, which at first becomes acid, and then strongly alkaline. They also occur on boiled potatoes, carrots, etc., where they form small lemon-yellow masses. The colouring matter

* Flügge, *Fermente und Mikro-Parasiten.* 1883.
† Zopf, *Die Spaltpilze.*
‡ Zopf, *Die Spaltpilze.*

soluble in water, insoluble in ether and alcohol, unchanged by alkalies, decolorised by acids. It is similar to yellow aniline colours both spectroscopically and in ordinary reactions.

Bacterium indicum (*Micrococcus indicus*, Koch. *Bacillus indicus*, Flügge).—Very short rods with rounded ends. In plate-cultivations on nutrient agar-agar, the colonies have a scarlet tint. They are round, ovoid, or spindle-shaped, and have characteristic granular margins (Fig. 82). Grown

FIG. 82.—BACTERIUM INDICUM; COLONIES ON NUTRIENT AGAR-AGAR, × 60.

upon nutrient agar-agar in a test-tube, the appearances are very characteristic. In a pure cultivation a brilliant, vermilion-coloured reticulated pellicle develops on the surface (Plate II., Fig. 1). In the track of the needle beneath the surface no pigment is formed (Plate XIII., Fig. 2). Cultivated in nutrient gelatine they liquefy the medium, and colour it crimson. The growth, of a darker crimson hue, subsides to the bottom of the tube. Upon sterilised potato they form a vermilion layer (Plate XV., Fig. 2).

Bacterium rubrum, Frank.—Minute motile rods, singly, in twos, and fours. They were observed on boiled rice, where they develop a brick-red pigment.

Bacterium prodigiosum (*Micrococcus prodigiosus, Bacillus prodigiosus, "Blood-rain," "Bleeding host"*).—Very short rods with rounded ends, and thread-forms, ·5—1 μ in width, forming at first rose-red, and then blood-red zooglœa. They grow luxuriantly when cultivated on sterilised potatoes (Plate IX., Fig. 1), and on the sloping surface of nutrient agar-agar (Plate II., Fig. 3). They appear occasionally on bread, boiled rice, and starch-paste, and more rarely on boiled white of egg and meat. Milk sometimes becomes coloured blood-red by the growth of this fungus, an appearance formerly attributed to a disease of the cow.

In Paris, in 1843, the fungus was peculiarly prevalent, attacking especially the bread produced in the military bakehouses.

The cells themselves are colourless. The colouring matter resembles fuchsine; it is insoluble in water, but soluble in alcohol. The addition of acids changes it to a carmine red, and of alkalies to a yellow colour.

Bacterium luteum (*Bacillus luteus,* Flügge).—Short immotile rods. Colonies irregular in form, appear brownish under a low power, but macroscopically yellow. In test-tube cultivations they form a yellow growth without liquefying the

gelatine. They occur contaminating plate-cultivations.

Bacterium violaceum, Bergonzini. — Cells similar to *Bacterium termo*, ·6—1 μ thick, 2—3 μ long. They occur on white of egg, forming a violet pigment.

Bacterium brunneum, Schröter. — Motile rods, producing a brown colour. They were observed on a rotting infusion of maize.

Bacterium fluorescens putidum (*Bacillus fluorescens putidus*, Flügge).—Short rods with rounded ends; motile; spore-formation not known. They form small dark colonies with a greenish sheen and penetrating odour. In test-tubes they produce a pale-grey turbidity, and after three days colour the medium with a greenish tinge spreading down from above. On potatoes they rapidly develop a brownish layer. They occur on decomposing substances, producing a greenish coloration.

Bacterium fluorescens liquefaciens (*Bacillus fluorescens liquefaciens*, Flügge).—Short rods with rounded ends. Colonies on plates develop an iridescence around them. In test-tubes a similar iridescent sheen is produced. On potatoes they develop a brownish layer.

Bacterium ureæ (*Micrococcus ureæ*, Cohn).—Cocci 1·25—2 μ in diam., singly or in chains, and rods. The rods split up by division into chains of cocci, and the latter are finally set free. The cocci

increase further by subdivision, and a jelly-like membrane develops around them. Masses of cocci exist in the form of irregular or roundish lumps. Cultivations, after twenty-four hours, consist exclusively of rods; after forty-eight hours, of cocci chains; and in fourteen days, of zooglœa; the cocci transplanted into fresh nourishing solution again grow into rods. These observations point to the existence of a pleomorphic species, *Bacterium ureæ*, and the former nomenclature *Micrococcus ureæ* must be regarded as untenable. They are ærobic; occurring in urine they set up ammoniacal fermentation, converting urea into carbonate of ammonia.* Rods, 2 μ long and 1 μ wide, have been isolated from stale urine (*Bacillus ureæ*, Leube), which also most energetically cause the ammoniacal fermentation of urine.

Bacterium aceti.—Cocci, short rods, long rods, leptothrix-forms, and zooglœa. Cocci and short rods may occur in the same thread. The long rods and threads may develop irregular swellings, so-called involution-forms, which have a thickened membrane and a grey colour. The effect of the action of this microbe is to oxidise alcohol in wine and other fruit juices into vinegar. The masses of zooglœa united together form a membranous layer which must not be mistaken for the pellicle formed by *Saccharomyces mycoderma*. The latter prepares the medium for the action of the *Bacterium aceti*.

* Zopf, *Die Spaltpilze*. 1885.

Bacterium Pasteurianum, Hansen. Morphologically similar to *Bacterium aceti*, but the cells contain a starch-like substance, which is turned blue by iodine. They occur in beer-wort.

Bacterium liodermos (*Bacillus liodermos*, Flügge. *Potato bacterium*). — Short rods with rounded ends, motile. On plate-cultivations the colonies appear as small white pellicles floating on liquefied gelatine. In test-tubes the gelatine is liquefied, and the growth sinks down in flocculent masses. They occur on potatoes, forming a smooth shining layer, which ultimately becomes crumpled.

Bacterium multipediculum (*Bacillus multipediculus*, Flügge).—Long slender rods. They form peculiar insect-like colonies on plate-cultivations. In test-tubes the appearance is less characteristic. They occur on potatoes, forming a dirty yellow growth.

Bacterium ramosum liquefaciens (*Bacillus ramosus liquefaciens*, Flügge).—Rods, slowly motile. They form characteristic colonies on plate-cultivations. The colonies gradually sink down, forming a well-marked funnel with later an appearance of concentric rings. In test-tubes the funnel-shaped liquefaction sends off rays into the surrounding gelatine. They occur occasionally, contaminating cultivations.

Bacterium Zopfii, Kurth.—Cocci, 1—1·25 μ in diameter; rods, and threads. Cultivated in a streak

on nutrient gelatine spread out on a glass slide, a peculiar development takes place. In twenty-four hours after inoculation threads have developed; in forty-eight hours, windings of the threads are observed, and in six days the threads have broken up into cocci (Fig. 83). They were observed in

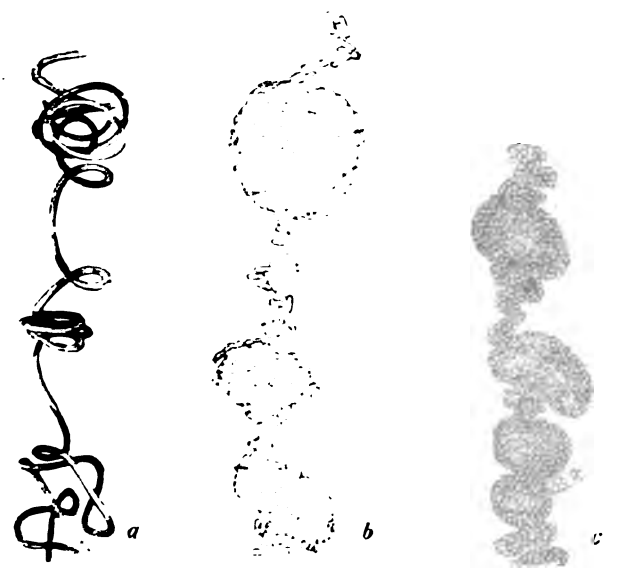


FIG. 83.—BACTERIUM ZOPFII. SUCCESSIVE CHANGES IN THE SAME THREAD, × 740: (*a*) A thread-form, (*b*) breaking up into rod-forms, (*c*) into cocci [after Kurth].

the intestine of fowls, especially in the contents of the vermiform appendix. Inoculation of rabbits was followed by negative results.

Bacterium merismopedioides, Zopf.—Forms threads 1—1·5 μ in thickness; these subdivide into long rods, short rods, and finally into cocci. The

cocci divide first in one and subsequently in two directions, forming characteristic groups, which appear like merismopedia. These groups may eventually consist of 64 × 64 cells or more, and ultimately form zooglœa. The cocci develop again into rods and threads. They were observed in water containing putrefying substances (River Panke, Berlin).*

Bacterium Pflügeri, Ludwig.—Large, round cocci, mostly in zooglœa, and thread-forms composed of rods. They can be cultivated on boiled white of egg and potatoes. They were observed to produce phosphorescence in putrid fish and meat.

Bacterium photometricum, Engelmann.—Cells slightly reddish in colour, motile. The movements are stated to depend on light.

Bacterium litoreum, Warming.—Cells ellipsoidal 2—6 μ long, 1·2—2·4 μ wide, occur singly in sea water, never as chains or zooglœa.

Bacterium fusiforme, Warming.—Cells spindle-shaped, with pointed ends, 2·5 μ long and ·5—·8 μ thick. Observed as a spongy layer on the surface of sea water.

Bacterium navicula, Reinke and Berthold.—Cells spindle-form or ellipsoidal, including motile and non-motile forms. They have one or more dark spots, which may be coloured blue by iodine. They have been observed in rotting potatoes.

Proteus vulgaris.—This and the two following

* Zopf, *Die Spaltpilze*. 1885.

species have been isolated* from putrefying meat infusion, and are stated to be intimately connected with the process of putrefaction. In the history of their development coccoid, bacterioid, spindle-form, spirulinar, and involution forms have been described. In *Proteus vulgaris* the bacteria vary in size; some measure 4 μ in length, and are almost as broad as long, and others vary from ·94—1·25 μ long and ·42—·63 wide. They are actively motile, and cultivated on nutrient gelatine they convert it into a turbid, greyish-white liquid. If cultivated in a capsule containing 5 per cent. of nutrient gelatine, a few hours after inoculation the most characteristic movements of the individual bacilli are observed on the surface of the nutrient gelatine, although at this early stage no superficial liquefaction can be detected. Probably the movements depend upon the existence of a thin liquid layer, as they are not observed if the nutrient medium contains 10 per cent. of gelatine.

Proteus mirabilis.—Cocci ·4 μ—·9 μ. They occur singly and in zooglœa, and sometimes in tetrads, pairs, chains, or as short rods in twos resembling *Bacterium termo*, in fact, in all conceivable transition-forms. Cultivated on nutrient gelatine they form a thick, whitish layer in concentric circles, which in time liquefies the medium. Similar movements are observed in capsule-cultivations as in *Proteus vulgaris*.

* Hauser, *Ueber Fäulniss-Bacterien*. 1885.

Proteus Zenkeri.—Cocci, ·4 μ in twos like *Bacterium termo*, and short rods 1·65 μ long. Cultivated on nutrient gelatine no liquefaction results, but a thick, whitish-grey layer is formed. The bacilli are motile, and the same phenomena are observed on the solid medium as in the other forms. In cover-glass impressions most varied groupings of the bacilli are seen, and also developmental and involution-forms.

The two following forms are only provisionally regarded as distinct species. They are both probably phase-forms of protean species.

Bacterium termo, Dujardin.—Short cylindrical or oblong cells, 1·5 μ long, ·5—·7 broad, generally occurring as dumb-bells. The cells have dark contents, invested by a thick membrane, and are provided with flagella, to which the characteristic movements are due (Plate I., Fig. 8). They are associated with putrefaction, invariably appearing in decomposing albuminous substances and liquids. A growth can be readily started by placing a piece of meat in water in a warm place. Cultivated in broth, they produce a turbidity, and on sterilised potatoes a slimy grey layer.

Bacterium lineola.—Cells 3·8 μ—5·2 μ long, 1·5 μ wide. They occur singly or in pairs, occasionally in zooglœa, but never in chains. The cells are provided with flagella, and contain strongly refringent contents. They resemble *Bacterium termo* in form and in movement, but are considerably larger. They occur in well water and stagnant water, and form slimy heaps on rotting potatoes, and zooglœa and pellicles on various infusions. Cultivated on nutrient agar-agar they form a semi-transparent growth (Plate XXIV., Fig. 1).

Genus II.—Spirillum.

SPECIES.

ASSOCIATED WITH DISEASE:—		
In man	Spirillum Obermeieri	Pathogenic.
	Spirillum Choleræ Asiaticæ	Pathogenic in man (?), possibly only saprophytic. Pathogenic in animals.
	Spirillum Finkleri	Saprophytic in man. Pathogenic in animals.
In animals	Spirillum tyrogenum	Saprophytic. Pathogenic in animals.
	Spirillum sputigenum	
UNASSOCIATED WITH DISEASE:—		
	Spirillum plicatile	Simple saprophytes.
	Spirillum serpens	
	Spirillum tenue	
	Spirillum undula	
	Spirillum volutans	
	Spirillum Rosenbergii	
	Spirillum attenuatum	
	Spirillum leucomelaneum	

Spirillum Obermeieri (*Spirochæte Obermeieri*, Cohn. *Spirillum of Relapsing Fever*).—Threads similar to the *Spirillum plicatile*. In length mostly 16—40 μ, with screw-curves regular (Plate I., Fig. 19). They move very rapidly, and exhibit peculiar wave-like undulations. They have been observed in the blood of patients suffering from relapsing fever,* but never in the secretions. They only occur during the relapses, and are absent during the non-febrile intervals. Their number is variable, but usually is strikingly great. Outside the body, in blood serum and 50 per cent. salt solution, the threads preserve their movements. From analogy to the *Spirillum plicatile* it is presumed that these threads are composed of articulated rods and cocci. Monkeys have been inoculated with success from

* Obermeier, *Med. Centralb.* 1873.

man,* but inoculations of mice, rabbits, sheep, and pigs gave negative results.

The spirilla were found in the blood of the inoculated monkeys in great numbers, and also in the brain, lung, liver, kidney, spleen, and skin; and are believed to be the cause of the disease.

METHODS OF STAINING THE SPIRILLUM OBERMEIERI.

In cover-glass preparations of blood the spirilla stain strongly with fuchsine, methyl-violet, gentian-violet, or bismarck-brown.

In sections, brown aniline stains have been recommended.

Spirillum choleræ Asiaticæ (*Comma-bacillus*, Koch).—Curved rods, spirilla, and threads (Plate I., Fig. 18). The curved rods or commas are about half the length of a tubercle-bacillus. They occur isolated, or attached to each other forming S-shaped organisms or longer screw-forms; the latter resembling the spirilla of relapsing fever. Finally they may develop into spirilliform threads. In old cultivations threads are found with bulgings or irregularities, which are called involution-forms (Plate I., Fig. 35).† The commas are actively motile; their movements and development into spirilla may be studied in drop-cultivations (Fig. 84). In plate-cultivations, at a temperature of from 16°—20° C., the colonies develop as little specks (Fig. 85), which

* Carter, *Lancet*. 1879 and 1880. Koch, *Cohn's Beiträge*.

† Compare also Van Ermengem, *Recherches sur le Microbe du Chol. Asiat.* 1885.

begin to be visible after about twenty-four hours. Examined with a low-power, and a small diaphragm, these colonies have the following characteristics. They appear as little masses, granular, and of a

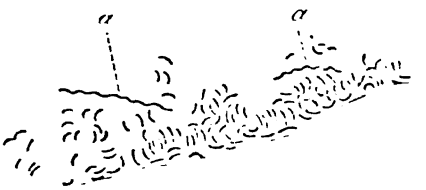


FIG. 84.—COVER-GLASS PREPARATION OF THE EDGE OF A DROP OF MEAT INFUSION, containing a pure cultivation of comma bacilli, with (*a*) spirilliform threads, × 600 [after Koch].

very faintly yellowish-red tinge, which have liquefied the gelatine, and sunk down to the bottom of the resulting excavations (Fig. 86).

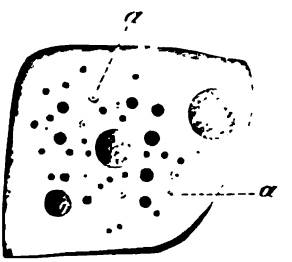


FIG. 85.—COLONIES OF COMMA BACILLI ON NUTRIENT GELATINE, NATURAL SIZE [after Koch].

In test-tubes of slightly alkaline nutrient gelatine (10 per cent.), the appearance of the growth is very striking. It commences to be visible in

about twenty-four hours. Liquefaction sets in very slowly, commencing at the top of the needle

FIG. 86.—COLONIES OF KOCH'S COMMA BACILLI, × 60; from a nutrient gelatine plate-cultivation.

track around an enclosed bubble of air, and forming a funnel continuous with the lower part of the

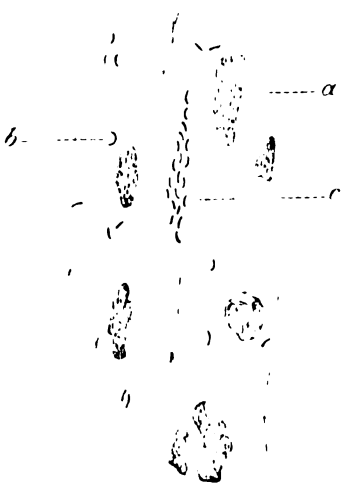


Fig. 87.

Fig. 88.

FIG. 87.—COVER-GLASS PREPARATION FROM THE CONTENTS OF A CHOLERA INTESTINE, × 600. (*a*) Remains of the epithelial cells; (*b*) Comma bacillus; (*c*) Group of comma bacilli [after Koch].

FIG. 88.—COVER-GLASS PREPARATION OF CHOLERA DEJECTA IN DAMP LINEN (two days old), × 600. Great proliferation of the bacilli with spirilla (*a*) [after Koch].

growth (Plate III., Fig. 1); the latter preserves for several days its resemblance to a white

thread (Figs. 92 and 93).* In about eight days, however, liquefaction takes place along the whole of the needle track.

On a sloping surface of agar-agar the cultivation develops as a white, semi-transparent layer, with well-defined margin. In potato-cultivations the microbe

FIG. 89.—SECTION OF THE MUCOUS MEMBRANE OF A CHOLERA INTESTINE, × 600. A tubular gland (*a*) is divided transversely; in its interior (*b*) and between the epithelium and the basement membrane (*c*) are numerous comma bacilli [after Koch].

will only grow at the temperature of the blood (37°C.), forming a slightly brown, transparent layer. Inoculation of a cultivation of the bacillus in the duodenum of guinea-pigs, with † and without ‡ ligation of the bile duct, has given positive results. More recently these results have been confirmed by the following method. Five ccm. of a 5 per

* From *Remarks on the Comma-Bacillus of Koch. Lancet.* 1885.
† Nicati et Rietsch, *Com. à l'Académie de Médecine.* 1884.
‡ Van Ermengem, *Le Microbe du Choléra Asiatique.* 1885.

cent. solution of potash were injected into the stomach of a guinea-pig, and twenty minutes after ten ccm. of a cultivation of comma-bacilli diffused in broth were similarly introduced. Simultaneously

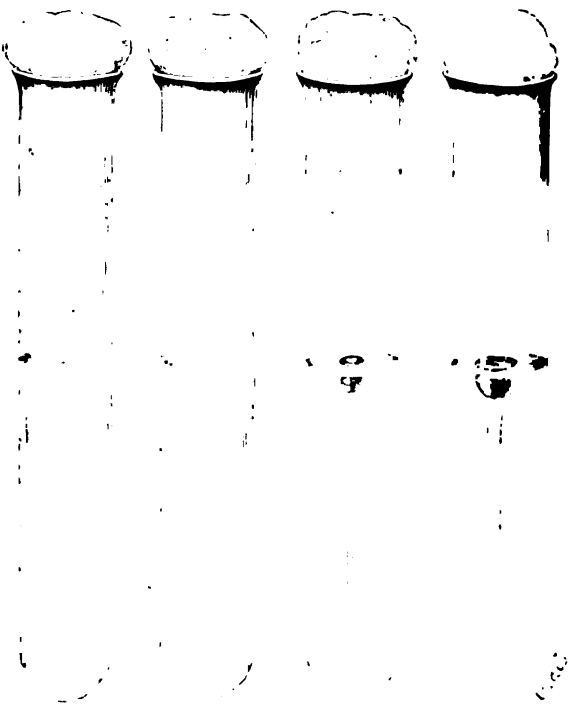


Fig. 90. Fig. 91. Fig. 92. Fig. 93.

PURE CULTIVATIONS IN NUTRIENT GELATINE.

Fig. 90. Finkler's bacillus, twenty-four hours old.
Fig. 91. ,, ,, two days old.
Fig. 92. Koch's cholera bacillus, twenty-four hours old.
Fig. 93. ,, ,, two days old.

with the latter, an injection of tincture of opium was made into the abdominal cavity, in the proportion of 1 ccm. for every 200 grammes' weight of the animal. Those who have had success with

inoculation experiments maintain that choleraic symptoms were produced without any trace of peritonitis or putrid infection, and that the comma-bacilli of Koch were again found in the intestinal contents, and fresh cultivations established.

On the other hand, these results have been disputed, the fatal effects of the inoculation attributed to septicæmic poisoning, and the proliferation of the bacilli considered to be dependent upon an abnormal condition of the intestines induced by the injection of tincture of opium.* It is, however, very probable that these organisms, like several others which have been isolated from intestinal discharges, are truly pathogenic in the lower animals. The comma-bacilli were found in the superficial necrosed layer of the intestine, in the mucous flakes and liquid contents of the intestinal canal of cases of Asiatic cholera † (Figs. 87, 88, 89). It is stated that they were also detected in a tank which contained the water supply to a neighbourhood where cholera

* Klein, *Brit. Med. Journal, and Micro-organism and Disease.* 1885. Lankester, *Nature*, xxxi.; *Nineteenth Century*. July, 1885. Klein and Gibbes, "An Inquiry into the Etiology of Asiatic Cholera." *Bluebook*, 1885.

† At a meeting of the Physiological Society, May 15th, 1886, at Cambridge, a preliminary communication was made upon the investigations in Spain referred to in the first edition of this work. The observations made by Roy, Brown, and Sherrington rather tend, in the opinion of the author, to confirm Koch's views. Comma-bacilli were found to be present, in some cases, in enormous numbers, and the frequency of their occurrence led these observers to believe that they must bear some relation to the disease. At the same time, as they failed to find them in all cases, they regarded the existence of a causal relation as not proven. They failed to find the Naples bacterium or the small straight bacillus noted by Klein; but they

cases occurred; but comma-shaped organisms are commonly present in sewage-contaminated water (Fig. 94). The comma-bacilli are ærobic, and their development is arrested by deprivation of oxygen. They are destroyed by drying and the presence of various antiseptic substances. They are distinguished from all other comma-shaped organisms by the test of cultivation. The entirely different results obtained in the case of the comma-bacilli of cholera nostras (Figs. 90 to 93), renders a thorough acquaintance with these bacilli of the greatest importance as an aid in diagnosis.

FIG. 94.—COMMA-SHAPED ORGANISMS WITH OTHER BACTERIA IN SEWAGE-CONTAMINATED WATER, × 1200.

drew attention to certain peculiar mycelium-like threads in the mucous membrane of the intestines. These organisms, however, judging from a preparation stained with methylene blue which was exhibited at the meeting, appeared to the author to much more closely resemble some of the involution forms of the comma-bacillus, *filaments à masses globuleuses*, figured by Van Ermengen, than anything else he had seen. Yet assuming these peculiar structures to belong as described to some species of Chytridiaceæ, it is very doubtful whether they can be considered to have any significance. Methylene blue has been employed by Koch and others, including the author, for staining sections of the intestine from cholera cases, and had they been constantly present it is hardly possible that such striking objects could have been overlooked. Again, we must bear in mind that hyphomycetous fungi occasionally have been found to occur saprophytically in the intestinal canal as well as in the lungs, external auditory meatus, and elsewhere. We must wait, before expressing a more definite opinion, until the report of these observers is published in full.

METHODS OF STAINING THE COMMA-BACILLI OF KOCH.

In cover-glass preparations they may be well stained in the ordinary way with an aqueous solution of methyl violet or fuchsine, or by the rapid method, without passing through the flame (p. 50, Babes' method).

Nicati and Rietsch's method.[*]

A small quantity of the stools or of the scraping of the intestinal mucous membrane is spread out on a glass slide and dried, then steeped during some seconds in sublimate solution or in osmic acid (1—100). It is then stained by immersion in fuchsine-aniline solution (1 or 2 grammes of Bâle fuchsine dissolved in a saturated aqueous solution of aniline, washed, dried, and mounted in Canada balsam.

In sections of the intestine their presence may be demonstrated by

(*a*) *Koch's method.*[†]

Sections of the intestine, which must be well hardened in absolute alcohol, are left for twenty-four hours in a strong watery solution of methylene blue, or for a shorter time if the colour solution is warmed. Then treated in the usual way.

(*b*) *Babes' method.*[‡]

Sections, preferably from a recent case of cholera, and made as soon as possible after death, are left for twenty-four hours in a watery solution of fuchsine (fabrique de Bâle), then washed in distilled water faintly acidulated with acetic acid, or in sublimate solution (1—1000), passed rapidly through alcohol and oil of cloves, dried with filter paper, and preserved in Canada balsam.

[*] *Brit. Med. Journal*, Sept. 1885.
[†] *Berliner Klinische Woch.*, No. 31.
[‡] Cornil and Babes, *Les Bactéries*, p. 458. 1885.

Spirillum Finkleri (*Comma-bacillus in Cholera nostras*).—Curved rods thicker than the comma-bacillus of Koch, and spirilla. The colonies on plate-cultivations (Plates VI. and VII.) are very much larger than those of the comma-bacillus of Koch of the same age. They have the faintest yellowish-brown tinge, a well-defined border, and a distinctly granular appearance. They liquefy nutrient gelatine very rapidly, so that the first plate of a series is, as a rule, completely liquefied on the day following inoculation, and the second plate in two or three days more. In a test-tube cultivation in nutrient gelatine the appearances are especially characteristic; the gelatine is very rapidly liquefied along the whole track of the needle, so that the cultivation resembles a conical sack, or the finger of a glove turned inside out (Figs. 95 and 96). On a sloping surface of nutrient

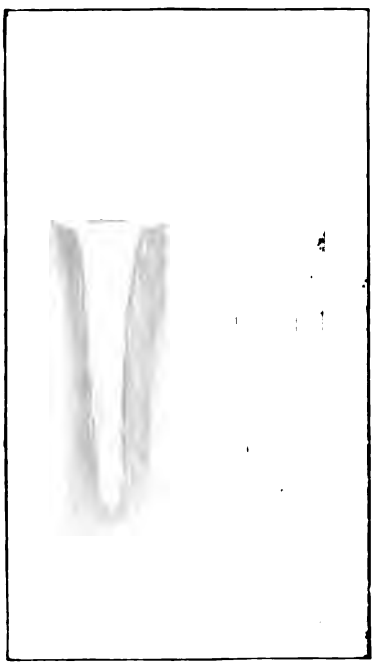


Fig. 95. Fig. 96.

PURE CULTIVATIONS OF THE SPIRILLUM FINKLERI IN NUTRIENT GELATINE.

Fig. 95. In twenty-four hours.
Fig. 96. In thirty-six hours.

agar-agar a white moist layer forms very quickly. On potatoes they grow at the ordinary temperature of the air, producing a brownish layer and corrosion of the surface of the potato. They were discovered in the evacuations of cases of cholera nostras, and were claimed at first to be identical with the comma-bacillus of Koch. By the test of cultivation they are now ascertained to be distinct. They also have been shown to be pathogenic.*

Spirillum sputigenum, Lewis.—Curved rods very similar to the comma-bacilli of Koch. Many

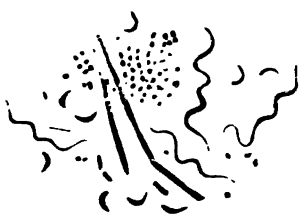


FIG. 97.—SPIRILLUM SPUTIGENUM. Occurring with spirochæta denticola, leptothrix-filaments, micrococci, and bacteria in a scraping from a carious tooth, × 1200.

have failed with repeated attempts to cultivate these bacilli, and, therefore, maintain that they are quite distinct biologically from the spirilla associated with Asiatic cholera. Others assert that they can be cultivated in an acid nutrient gelatine, and that they are identical with Koch's comma-bacilli in their mode of growth. They occur with other bacteria in saliva, and in scrapings from carious teeth (Fig. 97).

* Finkler and Prior, *Ergänzungshefte zum Centralblatt für Allgemeine Gesundheitspflege,* Erster Band. 1885.

Spirillum tyrogenum, Deneke.—Curved rods, slightly smaller than Koch's comma-bacilli, with a great tendency to form long spirillar threads (Fig. 98). The colonies on plate-cultivations are sharply defined, and of a greenish-brown colour. After a time they liquefy the gelatine, but the liquefaction is much more marked than in colonies of Koch's commas of the same age, though not so rapid as in the case of the commas of *cholera nostras*. In test-tubes of nutrient gelatine a turbid liquefaction occurs along the needle track, and on the surface of nutrient agar-agar a yellowish-white

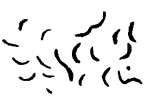


FIG. 98.—SPIRILLUM TYROGENUM. From a cultivation in nutrient gelatine, × 1200.

layer develops. Inoculation of potatoes gives no result. Administration of the bacilli by the mouth, in the manner employed for testing the pathogenic effect of Koch's bacilli, produced a fatal result in a few cases; on the other hand, injection into the duodenum failed entirely. The pathogenic properties may be, therefore, considered as not yet established. They were isolated from old cheese.

Spirillum plicatile, Ehrenberg (*Marsh-Spirochæte*).—Thin threads, 0·25 μ in breadth, with numerous narrow windings, 110—125 μ long, occurring also in spirulinar forms. The threads have primary and secondary windings; the former are in each example of equal size, but the latter are often

irregular; their ends are cut off bluntly, and they exhibit rapid movement. They occur abundantly in marsh-water in summer, and can be obtained by allowing algæ to decompose in water (Fig. 99). On cultivation the threads break up into long rods, short rods, and finally cocci. This change is rendered visible by making cover-glass preparations, and staining with aniline dyes.

FIG. 99.—SPIRILLUM PLICATILE (Marsh Spirochæte). From sewage-contaminated water, × 1200.

The following may be provisionally described as distinct species, though they are probably the spiral phase-forms of protean species.

Spirillum serpens, Müller (*Vibrio serpens*).—Threads 11—28 μ long, ·8—1·1 μ thick, with three or four windings. They are actively motile, often united into chains, or forming swarms, and are abundant in stagnant liquids.

Spirillum tenue.—Very thin threads, with at least 1½, usually 2—5 spirals. Height of a single screw is 2—3 μ, and the length of spiral, therefore, 4—15 μ. They are very swiftly motile, and often occur in felted dense swarms in vegetable infusions.

Spirillum undula.—Threads 1·1—1·4 μ thick, 9—12 μ long; spirals 4·5 μ high; each thread has 1½—3 spirals. They are actively motile, and possess at each end a flagellum. They occur in various infusions (Fig. 100).

Spirillum volutans, Ehrenberg.—Threads 1·5—

—2 μ thick, 25—30 μ long; tapering towards their extremities, which are rounded off. They possess dark granular contents. Each thread has $2\frac{1}{2}$—$3\frac{1}{2}$ windings or spirals, whose height is 9—13 μ. They have a flagellum at each end, and are sometimes motile, sometimes not. They are found in various infusions and water of marshes.

Spirillum Rosenbergii.—Threads with 1—$1\frac{1}{2}$ windings; 4—12 μ long; 1·5—2·6 μ thick. They are colourless, but the contents include strongly refractive sulphur granules. Also spirals 6—7·5 μ in height, which are actively motile, are found in brackish water.

FIG. 100.—SPIRILLUM UNDULA, × 1500.

Spirillum attenuatum, Warming.—Threads much attenuated at the ends, which consist usually of three spirals. The middle spiral is about 11 μ high, and 6 μ in diameter; and the end ones 10 μ high, and 2 μ in diameter. They are found in brackish water.

Spirillum leucomelaneum, Koch.—A rare form observed in water covering rotting algæ. Dark and glass-like spaces alternate in the spirillum, resulting from a regular arrangement of the dark granular contents.

Genus III.—Leuconostoc.

SPECIES.

UNASSOCIATED WITH DISEASE:—

Leuconostoc mesenteroides . . . Zymogenic saprophyte.

Leuconostoc mesenteroides, Cienkowski (*Gomme de sucrerie, Froschlaichpilz, Frogspawn fungus*).—Cells singly, in chains, and in zooglœa,

surrounded by a thick gelatinous envelope (Fig. 101). The life-history has been very thoroughly investigated.* The spores, 1·8—2 μ in diameter, are of a round or ellipsoidal form, with thick mem-

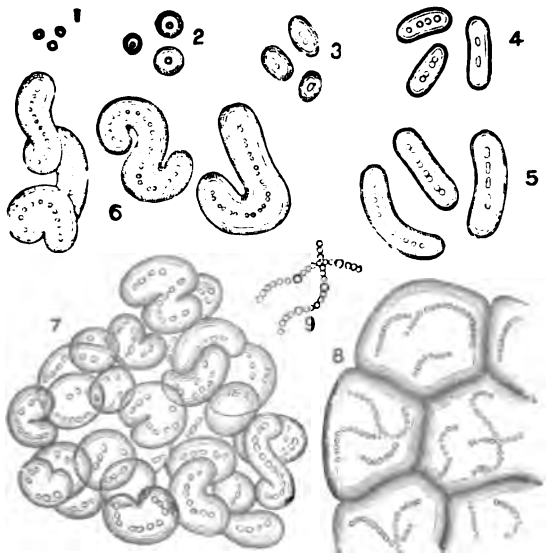


FIG. 101.—LEUCONOSTOC MESENTEROIDES.

1. Spores. 2. Spores after germination, showing gelatinous envelope. 3, 4, 5, 6. Increase by division. 7. Glomerular form of zooglœa. 8. Section of an old mass of zooglœa. 9. Cocci chains with arthrospores [after Tieghem and Cienkowski].

brane and shining contents. The outer membrane-layer bursts, and a middle lamella oozes out, and forms a thick gelatinous envelope, while the inner layer remains adherent to the plasma. Thus the spore-germination leads to the formation of a

* Cienkowski, *Die Gallertbildungen d. Zuckerrübensaftes*, 1878; and Van Tieghem, "Sur la Gomme de ucrerie," *Ann. Sc. Nat.* 1879.

coccus with a gelatinous envelope. The coccus then elongates into a short rod-form, and the gelatinous envelope becomes ellipsoidal. The rod divides into two cocci, and each of these lengthens into a rod and divides. By repetition of this process a chain of cocci results, encased in a cylindrical or ellipsoidal envelope. The chains increase in length, become twisted up, and eventually fall apart into pieces of various lengths. In nourishing liquids a great number of little masses are formed, which adhere together, and produce pseudo-parenchymatous structures. These latter may join together, forming still larger agglomerations. The masses of zoogloœa are of almost a cartilaginous consistency, and admit of sections being made with a razor. After a long time the envelope liquefies, and the cocci are set free; the latter introduced into fresh nourishing media develop new colonies. In the chains some of the cocci become enlarged without changing their form. These acquire the properties of spores, and are called *arthro-spores* (p. 131).

This micro-organism occurs occasionally in beet-root juice and the molasses of sugar-makers, forming large gelatinous masses resembling frog-spawn. The vegetation is so rapid that forty-nine hectolitres of molasses, containing 10 per cent. of sugar, were converted within twelve hours into a gelatinous mass; consequently, it is a formidable enemy of the sugar manufacturers.

Genus IV.—Bacillus.

SPECIES.

ASSOCIATED WITH DISEASE :—		
In man	Bacillus lepræ	Pathogenic.
	Bacillus in syphilis	Pathogenic (?) ; possibly only saprophytic.
	Bacillus typhosus	,, ,, ,,
	Bacillus malariæ	Pathogenic in man (?), pathogenic in animals.
	Bacillus of choleraic diarrhœa from meat poisoning	Pathogenic.
	Bacillus pyogenes fœtidus	Saprophytic in man, pathogenic in animals.
	Bacillus in septicæmia in man	Saprophytic.
	Bacillus in gangrenous septicæmia	,,
In animals	Bacillus tuberculosis	Pathogenic.
	Bacillus anthracis	,,
	Bacillus mallei	,,
	Bacillus of malignant œdema	,,
	Bacillus of septicæmia of mice	,,
	Bacillus of ulcerative stomatitis in the calf	,,
	Bacillus of swine-typhoid	,,
	Bacillus of swine-erysipelas	,,
	Bacillus in tetanus	Pathogenic (?)
	Bacillus alvei	Pathogenic.
UNASSOCIATED WITH DISEASE :—		
	Bacillus ianthinus	Chromogenic saprophytes.
	Bacillus pyocyaneus	
	Bacillus cyanogenus	
	Bacillus acidi lactici	Zymogenic saprophytes.
	Bacillus Fitzianus	
	Bacillus subtilis	Simple saprophytes.
	Bacillus figurans	
	Bacillus of jequirity	
	Bacillus caucasicus	
	Bacillus dysodes	
	Bacillus Hansenii	
	Bacillus erythrosporus	
	Bacillus septicus	
	Bacillus saprogenes	
	Bacillus fœtidus	
	Bacillus putrificus coli	
	Bacillus coprogenus fœtidus	
	Bacillus aerophilus	
	Bacillus mesentericus fuscus	
	Bacillus mesentericus vulgatus	

Bacillus lepræ, Hansen.—Fine slender rods, 4—6 μ long, and less than 1 μ wide, occasionally pointed at both ends, some clearly motile, and others not. In tissue sections they have a beaded

appearance (Fig. 102). Spore-formation has been described. They have been cultivated artificially on blood serum and alkaline meat extract. Inoculation experiments on monkeys and other animals have failed to produce the disease; though in cats and rabbits there have been indications of success.* The bacilli occur in enormous numbers in tubercular leprosy in the nodules of the skin (Plate XXIII., Figs. 1 and 2), and of the mucous membrane of the mouth, palate, larynx,

FIG. 102.—LEPROSY BACILLI FROM A SECTION OF SKIN, × 1200.

etc.† They occur also in the liver, spleen, testicles, lymphatic glands, and kidneys (Plate XX., Fig. 2); and in the interstitial tissue of the nerves in anæsthetic leprosy. They probably spread by the lymphatics, and are not found in the blood. In their behaviour to staining reactions they are similar to the bacillus of tubercle, except that they stain much more readily.

METHODS OF STAINING THE BACILLUS OF LEPROSY.

Cover-glass preparations may be made in the ordinary way, or by a special method, which consists in clamping a nodule with a pile-clamp, until a state of anæmia of the tissue is produced. On pricking with a needle or sharp knife a drop of clear fluid exudes, from which cover-glass

* Damsch, *Virchow's Archiv*, Bd. 92, Heft 1.

† Thin, *Med. Chir. Trans. Lond.*, 1883; *Brit. Med. Journal*, No. 129, 1884, and Steven, *Brit. Med. Journal*, No. 1281, 1885.

preparations may be made.* Cover-glass preparations and sections may be stained by Ehrlich's method (p. 50), or the latter by the following process:—

Method of Babes.†—Preparations are stained in a solution of rosaniline hydrochlorate in aniline-water. Decolorise in 33 per cent. hydrochloric acid, and after-stain with methylene blue.

Bacillus in syphilis, Lustgarten.‡—Rods resembling the bacilli of leprosy and tuberculosis, 3—4 μ long, ·8 μ thick. Two or more colourless, ovoid points in the course of the rod are visible with a high power; it is thought that they are possibly spores. The bacilli are always found in the interior of nucleated cells which are more than double the size of leucocytes. They have been observed in the discharge of the primary lesion, and in hereditary affections of tertiary gummata. Some observers state that an identical bacillus is found in normal secretions, § and others ‖ have described a bacillus associated with specific lesions, which is stated to differ from the above in its behaviour towards staining reagents.

METHOD OF STAINING THE BACILLUS OF SYPHILIS.

Method of Lustgarten:—

Sections are placed for from twelve to twenty-four hours in the following solution, at the ordinary temperature of

* Manson, *Lancet.* 1884.

† Babes, *Compt. Rend. de l'Acad. d. Sc.* 1883.

‡ Lustgarten, *Die Syphilisbacillen.* Mit 4 Tafeln. 1885.

§ Alvarez et Tavel, "Recherches sur le Bacille de Lustgarten," *Archiv de Phys. Norm. et Path.*, 17; Klemperer, "Ueber Syphilis und Smegma Bacillen," *Deutsche Med. Woch.* 1885.

‖ Eves and Lingard, *Lancet*, April 10th, 1886.

the room, and finally the solution is warmed for two hours at 60° C. :—

Concentrated alcoholic solution of gentian-violet .	11
Aniline water	100

The sections are then placed for a few minutes in absolute alcohol, and from this transferred to 1·5 per cent. solution of permanganate of potash. After ten minutes they are immersed for a moment in a pure concentrated solution of sulphurous acid. If the section is not completely decolorised, immersion in the alcohol and in the acid bath must be repeated three or four times. The sections are finally dehydrated with absolute alcohol, cleared with clove-oil, and mounted in Canada balsam.

Method of De Giacomi :—

Cover-glass preparations are stained with hot solution of fuchsine containing a few drops of perchloride of iron. They are then decolorised in strong perchloride of iron and after-stained with vesuvin or bismarck brown.

Method of Doutrelepont and Schütz :—

Sections are stained in a weak aqueous solution of gentian violet and after-stained with safranin.

Bacillus typhosus, Eberth (*Bacillus in typhoid fever*).—Rods, ·2 μ broad, and forming filaments up to 50 μ long ;* or† rods, short, rounded at their ends, and occasionally constricted in the middle; some exhibiting spore-formation. These bacilli have been observed in inflamed Peyer's glands, in the spleen, mesenteric glands, and the lungs in fatal cases of typhoid fever. More recently ‡ a bacillus has been cultivated on several plates of

* Kleb's *Arch. f. Experimental Pathol.* 1880.
† Eberth, *Virchow's Archiv*, Bd. 83.
‡ Gaffky, *Mittheil. a. d. K. Gesundheitsamte.* 1884.

gelatine which were inoculated from different spleens. After twenty-four hours the course of the inoculation streak became visible, and in forty-eight hours a distinct whitish growth had developed. With a low power this was found to consist of numerous colonies of a yellow-brownish colour. The gelatine was not liquefied. The rods varied in length

FIG. 103.—BACILLUS TYPHOSUS FROM A POTATO-CULTIVATION, × 1500.

(Fig. 103), were capable of development into threads, and were motile. They can be cultivated on potatoes at 37° C. They grow well also on blood serum, forming a whitish-grey, somewhat transparent layer. Spore-formation occurs at the ends of the rods. It is stated that inoculation experiments have been made in some cases with success.*

METHODS OF STAINING THE BACILLUS OF TYPHOID FEVER.

To stain the bacilli in the tissues the method of Gram can be employed, or the sections may be left for twenty-four hours in methylene-blue. Koch recommends bismarck-brown. To colour the spores cover-glass preparations and sections must be left for several days in the fuchsine solution employed in the method of Ehrlich (p. 163); or the solution may be warmed, and in the case of cover-glasses, even raised to boiling-point. They are then decolorised with nitric acid, and after-stained with methylene blue.

* Frænkel and Simmonds, *Die Ätiolog. bedeutung des Typhus-bacillus*. 1886.

Bacillus malariæ, Klebs (*Bacillus in intermittent fever*).—Rods, 2—7 μ long, which grow into twisted threads. Spore-formation takes place in the centre, or at either end (Plate I., Fig. 14).

Inoculated in rabbits they were stated to produce a febrile disorder analogous to malarial fever,* and in the spleen and marrow the threads and spores of the bacilli were found in abundance. Bacilli with end-spores have been discovered also in the blood of patients suffering from malaria.†

The bacilli were first described as present in the soil of the Roman Campagna.

According to more recent observations, peculiar amœboid bodies and motile filaments are constantly present in the blood of cases of malaria. These organisms, or *plasmodia malariæ*, appear to be closely allied to the flagellated protozoa (see Appendix B).

Bacillus of choleraic diarrhœa from meat-poisoning, Klein.‡—Rods from 3—9 μ in length, 1·3 μ wide, rounded at their extremities, singly or in chains of two. Spore-formation occurs, the spores being 1 μ thick, oval, and situated in the centre or at the end of the rod.

Feeding with the bacilli and inoculation produced positive results. At the autopsy, pneumonia, peritonitis, pleuritis, enlargement of the liver and spleen, and hæmorrhage were observed, and bacilli were

* Klebs and Tommasi Crudeli, *Archiv. f. Experimental Pathol.* 1879.

† Marchiafava, *ibid.*

‡ Klein, p. 87.

present in the blood and exudations of the animal. They occurred in the blood and juices, and especially in the glomeruli of the kidneys, of several fatal cases of choleraic diarrhœa.

Bacillus pyogenes fœtidus, Passet.—Small rods, with rounded ends of about 1·45 μ in length, and ·58 μ in width; often in twos, or linked together in chains. They are motile, and spore-formation occurs. When cultivated in nutrient gelatine, a greyish, veil-like growth forms on the surface. In plate cultivations white points appear after twenty-four hours, which develop into greyish spots, and these enlarging coalesce into a layer. In nutrient agar-agar the cultivation resembles the growth on gelatine. On blood serum a moderately thick greyish-white streak develops, and on sterilised potato an abundant, shining, brownish culture. From all these media a putrid odour emanates, but no smell is detected from a cultivation in milk. Inoculated into mice and guinea-pigs abscesses are produced or death from septicæmia results. They were isolated from putrid pus.

Bacillus in septicæmia of man, Klein.*—Rods singly or in chains, 1—2·5 μ long, ·3—·5 μ wide, which were observed in the blood-vessels of the swollen lymphatic glands. They are possibly identical with the following:—

Bacillus in gangrenous septicæmia, Arloing and Chauveau. — Short rods, possessing

* Klein, *Micro-organisms and Disease.* 1885.

spores, were observed around wounds in gangrenous septicæmia, and considered to be the cause of the gangrene.

Bacillus tuberculosis, Koch.*—Rods, 2—4 μ and occasionally 8 μ long, very thin, and rounded at the ends. They are straight or curved, and frequently beaded (Fig. 104), and occur singly, in pairs, or in bundles. They are found in the cells

FIG. 104.—BACILLUS TUBERCULOSIS, FROM TUBERCULAR SPUTUM, STAINED BY EHRLICH'S METHOD, × 2500. From photographs.

of tubercles, especially in the interior of giant cells. In the latter they are often accompanied with grains which exhibit the same colour reaction (Plate XVIII., Fig. 1). They are non-motile. Spore-formation has been described (see p. 134). The best medium for cultivation is solid blood serum of cow or sheep, with or without the addition of gelatine; and the most favourable temperature for their development is 37°—38° C. The

* Koch, *Berl. Klin. Woch.*, No. 15, 1882; and *Mittheil. aus dem Kaiserlich. Gesundheitsamte*, "Ætiologie der Tuberkulose."

growth takes place very slowly, and only between the temperatures of 30° and 41° C. In about eight or ten days the growth appears as little whitish or yellowish scales and grains (Plate XI., Fig. 1).

The bacillus can also be cultivated in a glass capsule on blood serum, and the appearances of the growth studied under the microscope. The scales or pellicles are then seen to be made up of colonies of a perfectly characteristic appearance, which may be still further studied by making a cover-glass impression (p. 52, and Plate XI., Fig. 4). They are then seen to be composed of bacilli, arranged more or less with their long axis corresponding with that of the colony itself, and with an appreciable interval between the individual bacilli. The colonies themselves appear as fine curved lines, the smallest being mostly S-shaped. Longer colonies have serpentine twistings and bendings, which often recall the curves of fancy lettering. The ends of the lines run to sharp points, but the middle of the growth is spindle-formed. The youngest colonies are extremely delicate and narrow, but the older colonies increase in size, are thicker across, and, blending with each other, gradually obliterate the characteristic appearances; a lamellated growth results, which increases, and gives the appearance to the naked eye of the scale or pellicle already described. The blood serum is not liquefied unless putrefactive bacteria contaminate the culture. A fresh tube can be inoculated with one of these little

scales, and a new generation started. The scales gradually increase in size, and consist entirely of bacilli. In about three to four weeks the cultivation ceases to increase, and it is then necessary to inoculate a fresh tube. The virulence is not weakened by carrying on successive cultivations. A relatively small portion of the cultivation inoculated into the subcutaneous tissue, into the peritoneal or pleural cavity, into the anterior chamber of the eye, or directly into the blood stream, produces after three or more weeks artificial tuberculosis in guinea-pigs and rabbits. Dogs and cats can also be infected by experimental inoculation.

The appearances observed at the autopsy are, swollen lymphatic glands in the neighbourhood of the inoculation, followed by softening and abscess; enlargement of the spleen and liver, with formation of caseous tubercles; tuberculosis of the lungs, bronchial glands, and peritoneum. After inoculation of the eye, grey tubercles appear on the iris, and undergo enlargement and caseation, followed by tuberculosis of the eyeball and organs generally. The bacilli appear to be the direct cause of tuberculosis, and the presence of the bacillus in the sputum of patients is regarded as a distinctive sign of the existence of this disease. The detection of the bacillus has, consequently, become a test which is daily applied by physicians in forming clinical diagnoses.

The bacilli are found in all tubercular growths

of man, monkeys, cattle (*Perlsucht*), birds, and many other animals, and in cases of artificial tuberculosis, in rabbits, guinea-pigs, cats, etc. (Plate XVIII., Fig. 2). In man the bacillus can be detected in the tissues, in the sputum, in the blood, and in the urine.*

Tuberculosis may also be produced by inhalation and feeding experiments (p. 107). The channels of infection in man are also most probably the pulmonary or intestinal mucous membranes. The possibility of inoculation of skin wounds is open to doubt. The bacilli or their spores are inhaled from the air, or taken in with food. As a relatively high temperature is required for their growth, they cannot thrive outside the animal body in cold climates. Morphologically identical bacilli have also been observed, but very sparsely, in sections of lupus.

METHODS OF STAINING THE TUBERCLE BACILLUS.

Numerous methods have been recommended for staining the *bacillus tuberculosis*, each of which will be given in detail

Ehrlich's and Gibbes' methods may be with advantage employed in staining cover-glass preparations. *Gibbes' rapid double stain* is obviously the best to employ for clinical purposes. For sections both *Ehrlich's and Neelsen's methods* give excellent results.

Koch's original method.—Cover-glass preparations or sections are laid in Koch's solution (No. 23, *c.*) for twenty-four hours, or for one hour if the solution is warmed to 40° C. Rinse in water; immerse in a watery solution of vesuvin for two minutes; rinse again in water, and examine; or, after rinsing in water, treat with alcohol, clove-oil, and Canada balsam.

* Babes, *Centralbl. f. d. Med. Wissensch.*, 1883, p. 145.

Ehrlich's method.—Cover-glass-preparations are allowed to float in a watch-glass, containing a solution of gentian-violet or fuchsine, added to aniline water. A saturated alcoholic solution of the dye is added till precipitation commences (10 ccm. aniline water, and 10—20 drops of the colour solution). The cover-glasses are left in the solution for about half an hour; then washed for a few seconds in strong nitric acid (one part commercial nitric acid to two of distilled water), and rinsed in distilled water. *After-stain* with vesuvin or methylene-blue, rinse in water, dry and preserve in Canada balsam (Plate XX., Fig. 1).

Sections and cover-glass-preparations may be stained by this method, as described by Koch.*

Saturated alcoholic solution of methyl-violet or fuchsine	11
Aniline water	100
Absolute alcohol	10

Preparations are left for twelve hours in this solution (colouring of the cover-glass-preparations can be expedited by warming the solution).

Treat the preparations with (1—3) solution of nitric acid a few seconds.

Wash in alcohol (60 per cent.) for a few minutes (cover-glass-preparations need only be rinsed a few times). After-stain with diluted solution of vesuvin or methylene-blue for a few minutes.

Wash again in 60 per cent. alcohol, dehydrate in absolute alcohol. Clear with cedar-oil, mount in Canada balsam.

Rindfleisch's method.—Prepare a solution composed of

Saturated alcoholic solution of fuchsine	10 drops
Aniline water	2 drams.

Pour it into a watch-glass, and float the cover-glass; warm the watch-glass over a spirit-lamp until steam rises. Remove it from the flame, and set it aside for five minutes.

* *Mittheil. aus dem Gesundheitsamte*, Zweiter Band, 1884, p. 10

Take out the cover-glass, and transfer it for a few seconds to acidulated alcohol (two drops of nitric acid in a watch-glass full of alcohol). Wash in distilled water, dry, and preserve in balsam. After-stain, if necessary, with bismarck-brown, or methylene-blue.

Weigert-Ehrlich method (*vide* p. 61).

Orth's modification of Ehrlich's method.—Stain by the method of Ehrlich, but decolorise with acidulated alcohol (one of hydrochloric to one hundred parts of 70 per cent. alcohol).

*Gibbes' method.**—Stain cover-glass-preparations in magenta solution (No. 22) for 15—20 minutes. Wash in (1—3) solution of nitric acid, until the colour is removed. Rinse in distilled water. After-stain with methylene-blue, methyl-green, iodine-green, or watery solution of chrysoidin, five minutes. Wash in distilled water till no more colour comes away. Transfer to absolute alcohol for five minutes; dry, and preserve in Canada balsam. Leave sections in the stain for half an hour, then treat with nitric acid, and wash with distilled water. Transfer to methylene-blue till deeply stained, wash again in distilled water, and then in spirit. Pass through absolute alcohol and clove-oil, and preserve in Canada balsam.

Gibbes' new method.—Cover-glass-preparations are placed in the double staining solution (No. 16), which has been warmed in a test-tube, and, as soon as steam rises, poured into a watch-glass. They are allowed to remain for five minutes, and then are washed in methylated spirit till no more colour comes away, dried in the air or over a spirit-lamp, and mounted in Canada balsam. If the solution is used without warming, the cover-glasses must be left in it for an hour. Sections are treated on the same principles, but must be left in the solution for several hours. The crumpling of the sections by the action of nitric acid is avoided.

* Gibbes, *Practical Pathology*. 1883

Baumgarten's method.—Cover-glass-preparations of sputum are made as already described (p. 48), and immersed in a very dilute solution of potash (1—2 drops of a 33 per cent. solution of potash in a watch-glass of distilled water) The cover-glass is pressed down on a slide, and examined with a high power. The bacilli can be thus examined in the unstained condition, and to avoid any mistake from confusion with other species, the cover-glass can be removed, dried, passed through the flame, and stained with a drop of an aqueous solution of fuchsine, or gentian-violet. The putrefactive bacteria are stained, but the tubercle bacilli remain absolutely colourless.

Baumgarten's new method.—A solution is prepared as follows: Drop 4—5 drops of concentrated alcoholic methyl-violet solution into a small watch-glass full of water. (*a*) Stain the sections in this solution, wash them in water, and decolorise in absolute alcohol (five to ten minutes), or, before treating with alcohol, immerse the sections for five minutes in a half-saturated solution of carbonate of potash. Pass through clove-oil, and mount in a mixture of Canada balsam, free from chloroform, and clove-oil (equal parts). The object of this process is to differentiate the tubercle bacilli from chance bacteria, inasmuch as the tubercle bacilli gradually are decolorised by the clove-oil. (*b*) Sections stained in the above solution are placed for five minutes in alcohol, and then in a concentrated solution of bismarck-brown in 1 per cent. solution of acetic acid. The after-treatment may be conducted as already described.

Neelsen's method. — Cover-glass-preparations may be quickly stained in Neelsen's solution (No. 25) warmed in a watch-glass till steam rises. Sections are left for from five to ten minutes in the solution, and then washed in a watery solution of sulphuric acid (25 per cent.); rinsed in distilled water, and immersed in methylene blue solution. After two or three minutes they are passed through alcohol and oil of cloves, and mounted in Canada balsam.

Balmer-Fräntzel method.—Dissolve two grammes of freshly-powdered gentian-violet in 100 grammes of aniline-water. Immerse the sections for twenty-four hours, and treat as in Ehrlich's method.

Ziehl's method.—Stain with Ehrlich's method, but omit the nitric acid; after-stain with methylene-blue. The latter replaces the stain of all bacteria except the tubercle bacillus.

Lichtheim's method.—Concentrated solution of fuchsine or gentian-violet is diluted with distilled water, and the sections stained for thirty-six hours.

Peters' method.—Sections are stained for half an hour in fresh aniline-gentian-violet solution. Transfer to 20 ccm. of absolute alcohol for eighteen hours, the alcohol being renewed two or three times. Rinse in distilled water for one minute, and immerse for three minutes in a watery solution of aniline-yellow (aniline-yellow ·2 dissolved in distilled water 10, filter). Wash in absolute alcohol, clarify with clove-oil, and preserve in Canada balsam.

Fränkel's method.—Sputum preparations are rapidly double-stained by the following method: Prepare a solution by adding concentrated alcoholic methyl-violet or fuchsine solution, drop by drop till opalescence arises, to 5 ccm. of aniline-water heated to 100° C. Float the prepared cover-glasses two minutes in the warmed solution. The process of after-staining and decolorisation is effected by placing the preparation for one to two minutes in one of the following solutions: for fuchsine-stained preparations a saturated solution of methylene-blue in a mixture of

Alcohol	50
Distilled water	30
Nitric acid	20

which is filtered before use; for preparations stained in methyl-violet, a saturated solution of vesuvin may be used in

Alcohol	70
Nitric acid	30

which must be filtered before use. The sections are washed in water (or weakly acidified 50 per cent. alcohol), dried and mounted in the usual way.

Pfuhl-Petri's method.—The colouring solution consists of 10 ccm. of a saturated alcoholic solution of fuchsine added to 100 ccm. of water. Float the cover-glasses for two minutes in the solution heated till steam rises. Wash for one minute in glacial acetic acid, rinse in water, and after-stain in an alcoholic or watery solution of malachite green for a half or one minute. Rinse again in water. Dry, and examine in glycerine, or preserve in Canada balsam.

Senkewitsch's method.—Stain cover-glass-preparations in concentrated fuchsine solution. When strongly coloured, wash out the stain for one to two minutes in alcohol, to which one drop of nitric acid has been added for every 10 ccm. Rinse in water, dry, and mount in Canada balsam.

Kaatzer's method.—Place the cover-glass-preparations for twenty-four hours in a solution of over-saturated alcoholic gentian-violet, or, if warmed to 80° C., for three minutes. Decolorise in a solution consisting of

Alcohol 90 per cent. . . .	100 ccm.
Water	20 ccm.
Strong hydrochloric acid . .	20 drops.

Rinse in 90 per cent. alcohol, and after-stain with concentrated watery solution of vesuvin for two minutes; wash again in distilled water, dry, and mount in Canada balsam.

Ehrlich's method and eosin.—The author has found that after sections have been stained with methyl-violet and bismarck-brown by Ehrlich's method, as described by Koch (p. 163), they may with advantage be immersed in a weak alcoholic solution of eosin, then rinsed in clean absolute alcohol, clarified with clove-oil, and mounted in Canada

balsam. The giant cells are then stained pink, while their nuclei are brown and the bacilli blue (Plate XVIII., Fig. 1).

Bacillus anthracis (*Bactéridie du charbon, Bacillus of splenic fever, woolsorters' disease,* or *malignant pustule*).—Rods, 5—20 μ long and 1—1·25 μ broad, and threads; spore-formation present. As a thorough knowledge of the life-history of this bacillus is of the greatest importance, inasmuch as it is without any doubt the actual cause of widespread disease, the various steps to be followed in a practical study of it will be successively treated in detail. Its morphological and biological characteristics have been very completely worked out, and it serves as an excellent subject for gaining an acquaintance with the various methods that should be employed in studying micro-organisms. It is found that a mouse inoculated with the bacillus or its spores will die in from twenty-four to forty-eight hours, or more rarely in from forty-eight to about sixty hours.

Examination after death.—The details to be observed in the autopsy have already been described (p. 111). The spleen is found to be considerably enlarged, and may be removed (p. 112), and examined by making cover-glass preparations, inoculations, and subsequently sections.

Cover-glass preparations.—In cover-glass preparations of the blood of the spleen the bacilli are found in enormous numbers. Preparations should be

made similarly with blood from the heart and exudations from the lungs, etc. In the last-mentioned the bacilli are present in very small numbers, or altogether absent. They should be examined both un-

FIG. 105.—BACILLUS ANTHRACIS, × 1200. From a preparation of blood from the spleen of an inoculated mouse.

stained and stained (p. 48). The rods are straight, or sometimes curved, rigid, and motionless, and vary in size in different animals. They stain intensely with aniline dyes, and are then seen to be composed of segments with their extremities truncated at right

FIG. 106.—BACILLUS ANTHRACIS. × 1200. From a cover glass preparation stained with Gram's method and eosin. The light part of the rod represents the sheath stained pink, and the dark part the protoplasmic contents stained blue and contracted.

angles; between the segments a clear linear space exists, which gives them a characteristic appearance (Fig. 105). By double staining (p. 49), the rods are seen to consist of a membrane or hyaline sheath with protoplasmic contents (Fig. 106).

Drop-cultures.—A little of the blood from the spleen or heart is employed to inoculate the liquid medium, bouillon or blood serum. Several of these cultures should be prepared, and some of them placed in the incubator. Examined from time to time it will then be observed that the rods grow into long homogeneous filaments, which are twisted up in strands, and then untwisted in long and graceful curves. In a few hours they begin to swell, become faintly granular, and finally, bright, oval spores develop (Plate I., Fig. 28). The cultures in the incubator develop rapidly, a temperature of 25°—40° C. being most favourable for the growth of the bacillus. The spores are eventually set free, and by making a fresh cultivation, or by injecting them into a mouse or guinea-pig, they germinate again into the characteristic bacilli, which in their turn grow into filaments and spores. When the spore germinates it swells, the outer layer becomes jelly-like, and giving way at one or other pole, the contents escape and grow into a rod. With the precautions previously described (p. 112) cultivations should be established in nutrient gelatine, nutrient agar-agar, and on sterilised potatoes.

Test-tube cultivations in nutrient gelatine.—Typically characteristic appearances are obtained by inoculating a 5 to 8 per cent. nutrient gelatine. A whitish line develops in the track of the inoculating needle, and from it fine filaments spread out in the

gelatine * (Fig. 107). Occasionally a little isolated spot develops, from which rays extend in all directions, like the silky filaments of thistle-down. The filaments are more easily observed with a magnifying glass. In a more solid nutrient-gelatine the growth appears only as a thick white thread. As liquefaction of the gelatine progresses, these appearances rapidly disappear, and the growth subsides as a white flocculent mass (Plate V., Fig. 3). In exhausted culture-media, and sometimes in the blood, filaments are seen in a state of degeneration. This has also been observed in sections of the kidney, etc., of a rabbit inoculated with the anthrax bacillus, which had died of septicæmia the following morning.

FIG. 107. PURE CULTIVATION OF THE BACILLUS ANTHRACIS IN NUTRIENT GELATINE.

Test-tube cultivations in nutrient agar-agar.—Cultivated upon a sloping surface of nutrient agar-agar a viscous snow-white layer is developed (Plate XIV., Fig. 1). Without access of air no cultivation can be obtained, the bacilli being ærobic. This can be demonstrated by embedding a piece of lung or spleen pulp containing bacilli in nutrient agar-agar (p. 137). No growth of the bacilli takes place.

* The Author, *Lancet*, 1885.

Potato-cultivations.—A very characteristic growth results from the inoculation of sterilised potatoes. The damp-chamber containing the potatoes is placed in the incubator, and in about thirty-six to forty-eight hours a creamy, very faintly yellowish layer forms over the inoculated surface, with usually a peculiar translucent edge (Plate XV., Fig. 1). On removing the cover of the damp-chamber a strong, penetrating odour of sour milk is encountered.

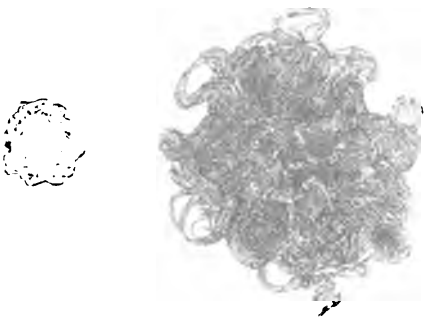


FIG. 108.—COLONIES IN A PLATE-CULTIVATION, × 70.

Plate-cultivations.—From the spleen or blood of the heart, cultivations must be established in nutrient gelatine on plates. The colonies develop in about two days, according to the temperature of the room. They appear to the naked eye as little white spots or specks, which, on examination with a low power of the microscope and small diaphragm, exhibit two distinct forms. One form, on careful focussing, has the appearance of a little compact ball of twisted thread; in the other, liquefaction of the gelatine has commenced, and the thread

bundles are spreading out like locks or plaits of hair in the neighbouring gelatine. These appearances are perfectly characteristic (Fig. 108).

Cover-glass impressions. — The plate-cultivations should be also examined as soon as the colonies appear, by making cover-glass impressions (p. 52), and staining them with aniline dyes. The filaments, examined with a high power, will then be seen to consist of a number of rods or segments (Plate I., Fig. 30). On the other hand, filaments from a

FIG. 109.—COVER-GLASS IMPRESSION-PREPARATION, × 70.

tube cultivation in a solid medium will be found to be composed, not only of rods, but here and there of torula-like involution-forms (Plate I., Fig. 30). In a cover-glass impression from a potato-culture (Plate I., Fig. 29) the individual segments have a great tendency to be isolated one from the other, and there is copious spore-formation.

Preservation of spores.—Spores may be preserved simply by allowing anthracic blood to dry and sealing it in a tube. The spores from a potato

cultivation are treated as follows:—The inoculated surface containing the creamy cultivation is sliced off in a thin layer, and is mashed up with distilled water in a glass capsule. Sterilised silk-thread is cut up into lengths of about a quarter of an inch, and allowed to soak in the paste for some hours, under a bell-glass. The threads are then picked out with a pair of forceps, and laid upon a sterilised glass plate, covered with a bell-glass, and allowed to dry. From the plate, when perfectly dry, they are transferred to a small test-tube, which can be plugged with cotton-wool, or sealed in the Bunsen burner.

Examination in the tissues.—The organs must be hardened in absolute alcohol, cut and stained, (pp. 54, 291). The method of Gram is the most instructive, and eosin a very satisfactory contrast stain. The capillaries all over the body, lungs, liver, kidney, spleen, skin, mucous membrane, etc., will be found to contain bacilli. In some cases the bacilli are so numerous (*e.g.*, in the capillaries of the kidney, Plate XVI., Fig. 2), that examination with a low power gives the appearance of an injected specimen.

Inoculation of animals.—A thread containing spores, a drop of blood from an infected animal, or a minute portion of a cultivation, introduced under the skin of a mouse or guinea-pig, causes its death, as a rule, in from twenty-four to forty-eight hours. Sheep fed upon potatoes which have been the

medium for cultivating the bacillus, die in a few days. Goats, hedgehogs, sparrows, cows, horses, are all susceptible. Rats are infected with difficulty. Pigs, dogs, cats, white rats, and Algerian sheep have an immunity from the disease. Frogs and fish have been rendered susceptible by raising the temperature of the water in which they lived.

Dissemination of the disease and mode of infection.—It has been stated that when carcases of animals which have died of anthrax are buried under the soil, the development of the bacilli into spores can take place. The spores were supposed to be taken up by earth worms, carried to the surface, and deposited in their castings; animals then grazing or sojourning on the soil are thus liable to be infected.* This has not been borne out by experiment.† Bacilli, however, occur in large numbers in the blood and discharges from the nose and mouth of the moribund animals, and in the urine and fæces. They find a nourishing soil in decaying vegetable and animal matter, and having free access of oxygen form copious spores, so that the grass is extensively contaminated.

In warm and marshy districts the spore-formation is still more active, and the spores may be carried by floods over adjacent meadows. As to the mode of infection, the animals may be directly infected through buccal wounds caused by siliceous grasses, or by wounds of insects; the intestinal and pul-

* Pasteur, *Bulletin de l'Académie de Médecine.* 1880.
† Koch, *Mittheil. a. d. Gesundheitsamte.* 1881

monary mucous membranes are also regarded as pathways of infection. In animals the disease is known as "splenic fever."

In man the mode of infection is by inhalation of spores, and ingress by the pulmonary or intestinal mucous membrane, or by direct inoculation of a wound or abrasion. The spores are derived from the wool or hides of animals which have died of anthrax, and the resulting disease is known as "wool-sorter's disease," and "pustula maligna." Bacilli are found in the serum of the pustule, and in sputum, urine, fæces, and sweat; and if the disease prove fatal, in the capillaries throughout the body.

Attenuation of the virus.—By cultivating the bacillus in neutralised bouillon at 42°—43° C. for about twenty days, the infecting power is weakened, and animals inoculated with it (*premier vaccin*) are protected against the disease.* To obtain a still more perfect immunity, they are inoculated a second time with material (*deuxième vaccin*) which has been less weakened. The animals are then protected against the most virulent anthrax, but only for a time. From such a culture, however, new cultures of virulent bacilli can be started, and a culture that is "vaccin" for sheep kills a guinea-pig, and then yields bacilli that are fatal to sheep.† Exposure to a temperature of 55° C., or treatment with ·5 to 1 per cent. carbolic acid, deprives the

* Pasteur, *Compt. Rend.*, 1861, and *Revue Scientifique*, 1883.
† Klein, *Micro-organisms and Disease.* 1885.

bacilli of their virulence. The virulence of the bacillus is also altered by passing the bacillus through different species of animals. The bacillus of sheep or cattle is fatal when re-inoculated into sheep or cattle; but, if inoculated in mice, the bacilli then obtained lose their virulence for sheep or cattle; only a transitory illness results, and the animals are protected for a time against virulent anthrax.* The possibility of mitigating the virus

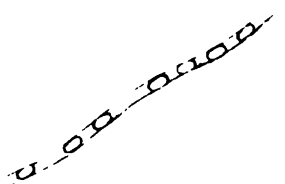


FIG. 110.—SPORES OF BACILLUS ANTHRACIS UNSTAINED, × 1500.

depends upon the species of animal; rodents cannot be rendered immune by any known "vaccin."

METHODS OF STAINING THE BACILLUS ANTHRACIS.

Cover-glass preparations of blood, etc., can be stained with a watery solution of any of the aniline dyes. They may be rapidly stained with a drop of fuchsine or gentian violet (p. 48), but more satisfactorily by floating the cover-glasses for twenty-four hours. The preparations may be dried and mounted in Canada balsam, but the typical appearances are best observed in freshly-stained specimens examined in water.

The sheath and protoplasmic contents can be differentiated by staining with eosin after the method of Gram.

* Klein, *Reports of the Medical Officer of the Local Government Board.* 1882.

The spores (Fig. 110) are not stained by the ordinary methods. The cover-glass preparations must be raised to a high temperature in the incubator, or treated with sulphuric acid (p. 309), or passed about twelve times through the flame of the Bunsen burner, or floated on hot solution of the dye.

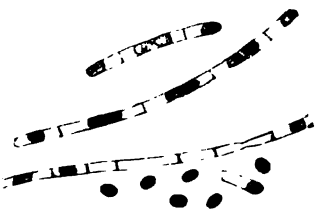


FIG. 111.—SPORES OF BACILLUS ANTHRACIS, × 1200; stained with gentian violet, after passing the cover-glass twelve times through the flame.

To double-stain spore-bearing bacilli.—Float the cover-glasses for about twenty minutes on hot alcoholic solution of fuchsine. Decolorise in weak hydrochloric acid, and after-stain with methylene blue (Fig. 112).

Tissue sections are best stained by the method of Gram,

FIG. 112.—FROM A DOUBLE-STAINED PREPARATION OF BACILLUS ANTHRACIS, × 1200.

and *after-stained* with eosin, picrocarminate of ammonia, or picro-lithium-carmine.

A more rapid double stain is obtained by immersing the sections in a watery solution of gentian-violet, rinsing in alcohol, and then staining by the method of Orth (p. 61).

Weigert's Method.—Place the sections for two to five minutes in a 1 per cent. watery solution of gentian violet.

Wash in alcohol, rinse in water, and transfer to picro-carmine solution (Weigert) for from half an hour to an hour. Treat with alcohol till the colour is almost washed out, and finally clear in oil of cloves and mount in Canada balsam.

Bacillus mallei (*Bacillus of glanders*).—Rods about the size of tubercle bacilli (Fig. 113). When cultivated on solid sterile blood serum at 38° C., the growth appears in the form of minute, transparent drops consisting entirely of the characteristic bacilli. On sterilised potatoes they form, in a week to ten days at 37° C., a brown gelatinous layer. Pure cul-

FIG. 113.—BACILLUS MALLEI, × 1200; from a section of a glanders' nodule.

tivations after several generations produce the following results when inoculated into horses, rabbits, guinea-pigs, and field-mice. A spreading ulcer with indurated base appears at the site of inoculation, while smaller ulcers break out in its vicinity. The lymphatics become swollen, and general infection follows in the form of nodules in the internal organs, and nodules and ulcers on the nasal septum. In guinea-pigs a characteristic tumour of the testis, or ovary and vulva, frequently results, and should be prepared for microscopical sections. The bacilli are found in the nodules of the nasal mucous mem-

brane, the lung, spleen, liver, and other organs in horses and sheep affected with glanders.

METHODS OF STAINING THE BACILLUS OF GLANDERS.

The bacilli of glanders are extremely difficult to demonstrate. The most satisfactory results are obtained as follows :—

Method of Schutz.—The sections are placed for twenty-four hours in a mixture of

Potash solution (1 in 10,000); } Equal
Concentrated alcoholic methylene-blue solution; } parts.

Wash the sections in a watch-glass with water acidulated with four drops of acetic acid. Transfer for five minutes to 50 per cent. alcohol, fifteen minutes to absolute alcohol, clarify in clove-oil, and mount in Canada balsam.

Bacillus œdematis maligni, Koch (*Pasteur's Septicæmia*). Rods from 3—3·5 μ long and 1—1·1 μ wide; they mostly lie in pairs, and then appear to be double this length. The rods are rounded at their ends, and form threads which are sometimes straight, but more commonly curved. In stained preparations they have a somewhat granular appearance. The bacilli are distinguished from anthrax bacilli by their being somewhat thinner, by their rounded ends, and by their being motile. Anthrax bacilli also never appear as threads in fresh blood, and are differently distributed throughout the body. They are anærobic, and can be cultivated on blood-serum and on neutral solution of Liebig's meat extract in an atmosphere of carbonic

acid. By embedding material containing bacilli in nutrient agar-agar and nutrient gelatine, characteristic cultivations are obtained. The following process may be adopted to obtain a pure cultivation.* A mouse inoculated subcutaneously with dust, as a rule, dies in one to two days. It is then pinned out, back uppermost, on a slab of wood (p. 113), and the hair singed with a Paquelin's cautery from one hind leg up to the neck, across the latter, and down again to the opposite hind leg. Following the cauterised line, the skin is cut through with sterilised scissors, and the flap turned back and pinned out of the way. With curved scissors little pieces of the subcutaneous œdematous tissue, in the neighbourhood of the inoculated spot, are cut out, and sunk with a platinum needle in a 1 per cent. nutrient agar-agar, or 5 per cent. nutrient gelatine. Fragments of tissue may also be embedded by the method already described (p. 137).

The inoculated tubes are placed in the incubator. In a few hours a whitish turbidity spreads out from the piece of tissue, and upwards in the needle track. Examined microscopically, the turbidity is found to be due solely to the development of bacilli of œdema. The surface exposed to the air exhibits no trace of the bacilli.

To investigate the tubes microscopically, a sterilised glass tube with a capillary end may be used,

* Hesse, *Deutsch. Med. Woch.*, No. 14. 1885.

with its neck plugged with sterilised cotton wool, and provided at the mouth with a suction ball. The capillary end is thrust into the cultivation, and a small fragment removed by aspiration. In the course of the first day the bacilli spread throughout a great part of the agar-agar in such a way that a more or less equally diffused cloudiness of the medium ensues, with subsequent appearance of strongly marked clouds or lines of turbidity. At the same time gas bubbles develop along the needle track, and a collection of liquid takes place, while spore-formation also commences. The following day these appearances are more marked, the opacity is more pronounced, the development of gas increases, and the liquid contains more spore-forming bacilli and numerous free spores.

The nutrient gelatine cultures during the first day show no macroscopic change, but after a few days the piece of tissue is surrounded with a white halo. This gradually spreads in all directions, and is apparently beset with hairs. The gelatine liquefies, and the fragment of tissue, degenerated bacilli, and spores, sink to the bottom. The cultivation is also very characteristic in ½ per cent. nutrient agar-agar. If placed in the incubator, in a few hours a cloudiness forms around the piece of embedded tissue, which is caused by bacilli gradually spreading in all directions in the nutrient medium. Mice inoculated from these cultivations die more quickly than from the original infection from dust. On potatoes

they are cultivated by introducing a piece of liver or other tissue containing the bacilli, into the interior of a sterilised potato (p. 113), incubated at 38° C. The bacillus is not deprived of its virulence by cultivation. The spores of the œdema-bacilli appear to be very widely distributed. They are found in the upper cultivated layer of the soil, in hay dust, in decomposing liquids, and especially in the bodies of suffocated animals, which are left to decompose at a high temperature. From any of these sources animals can be successfully inoculated. If a guinea-pig, for example, be subcutaneously inoculated with earth, putrid fluid, or hay dust, death frequently occurs in from twenty-four to forty-eight hours. At the autopsy the most characteristic symptom is a widespread subcutaneous œdema, which originates from the point of inoculation, accompanied with air-bubbles, and contains a clear reddish liquid full of motile and non-motile bacilli. The internal organs are little changed, the spleen is enlarged and of a dark colour, and the lungs are hyperæmic, and have hæmorrhagic spots. Examined immediately after death, few or no bacilli are detected in the blood of the heart, but in that of the spleen, liver, lungs, and other organs, in the peritoneal exudation, and in and upon the serous coating of abdominal organs they are present in large numbers. If, on the other hand, the animal is not examined until some time after death, then the bacilli are found in the blood of the heart, and distributed all over the body.

Bacillus of septicæmia of mice, Koch.—Extremely minute bacilli, ·8—1 μ long, and ·1—·2 broad, often in pairs, seldom in chains of four. On cultivation they do not appear to make threads, but the bacilli lie together in masses. Spores have been observed. The bacilli are probably non-motile. They are most commonly in the interior of white blood corpuscles. In these they increase, and in many cases a white cell is only represented by a mass of bacilli. The bacilli, or rather their spores, occur in putrid liquids. If a number of mice are inoculated with a minimum quantity of putrid fluid, about a third of them die of septicæmia. They rapidly sicken, their eyes inflame, their eyelids stick together, they become soporific, and die in from about forty to sixty hours. At the autopsy one finds slight œdema at the seat of inoculation, and enlargement of the spleen; the bacilli are found both free and lodged in the white corpuscles, in the œdematous tissue, and in the blood capillaries. A minimal quantity of this blood produces the disease if inoculated in house-mice or sparrows. Field-mice have an immunity. Rabbits and guinea-pigs inoculated in the ear suffer from only a local erythema, which disappears after five or six days, and renders them for a time immune. Rabbits inoculated in the cornea suffer from an intense inflammation of the eyes. The bacilli are easily cultivated outside the body on a mixture of aqueous humour and gelatine, and

especially on nutrient gelatine rendered slightly alkaline with sodium phosphate. They grow also very well on the ordinary nutrient gelatine, forming in plate-cultivations scarcely perceptible cloud-like specks, and in a test-tube of nutrient gelatine they form a delicately clouded cultivation along the needle track (Figs. 114 and 115). A small quantity of pure cultivation carried through many generations reproduces the disease when inoculated into mice. The organs should be hardened in absolute alcohol, and sections stained preferably by the method of Gram (Plate XXII., Figs. 1 and 2).

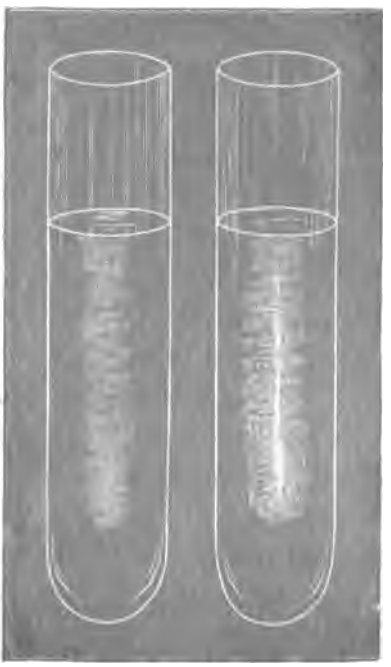


Fig. 114. Fig. 115.

PURE-CULTIVATIONS OF THE BACILLUS OF SEPTICÆMIA OF MICE IN NUTRIENT GELATINE.

Fig. 114. In two days.
Fig. 115. In five days.

Bacillus of ulcerative stomatitis in the calf, Lingard and Batt.—Rods 4 μ—8 μ, or more in length; 1 μ in width. Spores are frequently present. Injected into the rabbit or mouse they produce a fatal result. They were observed in ulcerations on the tongue and mucous membrane of the mouth of calves.

METHOD OF STAINING THE BACILLI OF ULCERATIVE STOMATATIS.

Sections through the ulcerations of the calf's tongue, or of the inoculated tissue of the rabbit, were stained by immersion in a mixture of magenta and methylene-blue. They were then washed in spirit, cleared in clove-oil, and mounted in Canada balsam.

Bacillus of swine-typhoid (*Bacillus of swine-plague or swine-fever. Bacillus of pneumo-enteritis of*

FIG. 116.—FROM A PREPARATION OF BRONCHIAL MUCUS OF A PIG [after Klein].

the pig, Klein*).—Rods 2—3 μ long, actively motile (Fig. 116); spore-formation described (Fig. 118). They can be cultivated in broth and hydrocele-fluid, and carried on through successive generations. A drop of any of these cultivations produces the disease in pigs, mice, and rabbits (Fig. 117); the animals die with a characteristic swelling of the spleen, coagulative necrosis of tracts of the liver tissue, and inflammation of the

* Klein, *Report to Med. Offic. Loc. Govt. Board.* 1877—1878.

lungs ; pigs inoculated with artificial cultures are protected against a fatal attack.

The bacillus was observed in the diseased organs

FIG. 117.—BLOOD OF FRESH SPLEEN OF A MOUSE, AFTER INOCULATION WITH SWINE FEVER [after Klein].

of pigs that had died of swine fever, and of animals that had died from the inoculated disease.

FIG. 118.—BACILLI FROM AN ARTIFICIAL CULTURE, WITH SPORES [after Klein].

Bacillus of swine-erysipelas (*Bacillus des erysipelas malignum, of Rothlauf, or Rouget du porc*). Extremely minute bacilli, bearing a close resemblance to the bacilli of septicæmia of mice.* In test-tubes of nutrient gelatine they develop a cloudy growth in the needle track (Plate XXVI.,

* Löffler und Schütz, *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, vol. i. 1885.

Fig. 1), and in plate-cultivations characteristic, thread-like, branching, or star-like colonies are formed (Plate XXVI., Fig. 2). Inoculated into mice and rabbits, a fatal result is produced; but experiments with pigs were unsuccessful. Pigeons were also susceptible, and the bacilli were detected in their blood (Plate XXVI., Fig. 3).

Bacillus in tetanus, Nicolaier.—Rods, somewhat longer but scarcely thicker than the bacillus of mouse-septicæmia. Occasionally thread-forms result, but they are collected mostly in irregular masses. They exhibit a characteristic spore-formation. They were found associated with other bacteria in abscesses resulting from the inoculation of mice and rabbits with garden earth. Inoculation of earth subcutaneously in these animals induces fatal tetanus. A cultivation of the mixture of micro-organisms on blood serum also produced the same disease. Bacilli stated to be identical with the bacilli of earth-tetanus have been observed in a case of tetanus in man. Further researches are required to establish their pathogenic properties.

Bacillus alvei, Cheshire and Cheyne.*—Rods varying in size, and forming large oval spores. When cultivated in nutrient gelatine in test-tubes a delicate, ramifying growth appears on the surface, and irregular whitish masses arise along the

* Cheshire and Cheyne, *Journ. Royal Microscopical Society*, 1885, pp. 582-601.

needle track. Processes shoot out from these masses, and extend through the gelatine for long distances. They are thickened at points in their course, and clubbed at the ends. The gelatine is gradually liquefied, and the bacilli form a loose, white, flocculent deposit at the bottom of the tube. The liquid in the tube becomes yellowish in colour after a time, and gives off an odour of stale but not ammoniacal urine. The colour and odour are distinctive also of the disease attributed to the bacilli. In plate-cultivations, the bacilli grow out in series of rods in single file, or in rows of several side by side. The processes which are formed, tend to curve, and at a short distance from the track of the needle-streak form a distinct circle, from which another process grows out, and a fresh circle is developed. The gelatine in the vicinity of the bacilli gradually liquefies, and channels are formed in the gelatine in which the bacilli move backwards and forwards. On nutrient agar-agar a whitish layer develops, consisting of bacilli arranged side by side, which in a few days are replaced by rows of spores similarly arranged. On potatoes they form a dryish yellow layer, and in milk a tremulous jelly. A cultivation of the bacillus in milk, sprayed over a honeycomb containing a healthy brood of bee larvæ, produced the disease known as "foul-brood." Adult bees fed on material containing bacilli became affected; inoculation of mice and rabbits with the bacillus gave

doubtful results. The bacilli were isolated from the diseased larvæ of bees.

Bacillus pyocyaneus (*Micrococcus pyocyaneus*, Gessard. *Bacterium æruginosum*, Schröter. *Bacillus fluorescens*).—Slender rods sometimes linked in twos or threes, or collected in irregular masses. Spore-formation present. On plate-cultivations white colonies with indistinct contour appear in twenty-four hours, and the whole of the gelatine has a greenish shimmer. In test-tube cultivations the gelatine is liquefied, and coloured green by reflected light, and a deep orange by transmitted light (Plate V., Fig. 1). On nutrient agar-agar they form a white layer, and colour the medium a pea-green. The pigment formed by the rods is a definite principle, pyocyanin.* It can be extracted with chloroform from pus, and from washings of bandages; it is soluble in acidulated water, which it colours red. In neutral solution it becomes blue. It crystallises in chloroform in long needles; and forms sometimes lamellæ and prisms. The rods occur in the pus of those cases in which the wounds and pus-stained bandages exhibit a greenish-blue colour.

Bacillus ianthinus (*Bacterium ianthinum*, Zopf. *Bacillus violaceus*).—Slender rods, about four times their width in length, with rounded ends. They also form threads, and are actively motile. Spore-

* Gessard, *De la Pyocyanine et de Son Microbe. Paris thèse.* 1882.

formation present in the rods. On plate cultivations the colonies occur as circumscribed liquefied areas, in the centre of which is a collection of the coloured growth. In test-tubes a funnel-shaped liquefaction takes place, while a granular-looking violet mass subsides to the bottom. On agar-agar and potatoes a beautiful violet growth rapidly develops. They were observed on pieces of pigs' bladder floating on the surface of water rich in bacteria. They occurred only on the surface of the bladder exposed to the air, and never on the part under water. They occasionally occur in common tap water. The colouring matter is soluble in alcohol.

Bacillus cyanogenus, Fuchs. (*Bacterium syncyanum. Bacillus of Blue Milk*).—Motile rods, 2·5—3·5 μ in length, and ·4 μ wide (Fig. 119). The rods after division may remain linked together, and form chains. Non-motile rods enveloped in a gelatinous capsule, and involution-forms, have also been described. Spore-formation present.

Cultivated in a test-tube of nutrient gelatine, the bacilli grow principally upon the free surface, in the form of a white layer. The surface of the gelatine becomes cupped, and a peculiar greenish-brown colour develops in the medium, especially in proximity to the growth.

On a sloping surface of nutrient agar-agar, they grow as a white layer, and colour the

upper part of the medium a smoky brown (Plate II., Fig. 2).

The bacilli can also be cultivated in milk and on various other substrata, as potatoes (Plate XXI.), boiled rice, and starch. A pure cultivation in neutralised sterile milk develops a weak alkaline

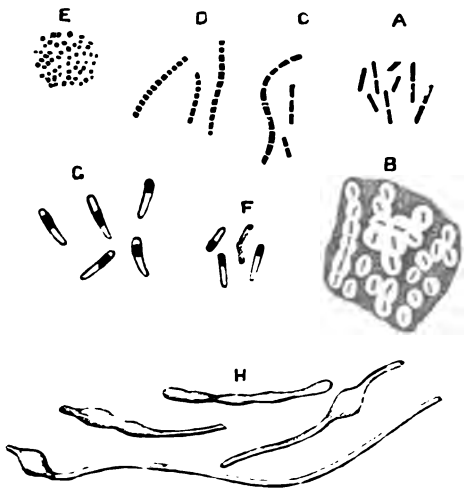


FIG. 119.—BACILLUS CYANOGENUS, × 650.

A. Active rods. B. Rods in zoogloea. C. Chain of short rods. D. Chain of cocci. E. Cocci stage. F, G. Spore-forming rods. H. Involution-forms [After Neelsen].

reaction. The colouring matter which is formed, varies with the nourishing medium; for example, in milk a slate-blue coloration is produced, but if the milk has become acid by the growth of the *Bacillus acidi lactici*, then the colour is an intense blue

The micro-organism occurs occasionally in

cow's milk, producing a blue colour. It has been observed, especially in the north of Germany, during the warm months; and where milk is kept in hot rooms, in the winter also. The blueing was originally attributed to a diseased condition of the cows, or to their eating certain meadow plants.

Bacillus acidi lactici.—Long and short rods, 1—2·8 μ long, ·3—·4 μ thick, and thread-forms; no cocci; spore-formation.* Cultivated on nutrient gelatine the breadth of the rods is lessened. They grow best between 35 and 42° C., and cease under 10° C. Cultivated at a temperature over 45·5°, they are no longer able to produce acidity. Probably several micro-organisms are able to produce an acid reaction in milk.

They occur with various other bacteria in sour milk, and a pure cultivation, isolated by plate-cultivations, turns sterilised milk sour.

Bacillus Fitzianus, Zopf.—Cocci, short rods, long rods, and threads. This bacillus, cultivated in meat extract and glycerine at 36° C., causes an active fermentation with the production of ethyl alcohol. Spore-formation occurs in the rods. Observed in unboiled hay infusion, accompanying the hay bacillus.

Bacillus subtilis (*Hay bacillus*).—Cylindrical rods as much as 6 μ in length, and about three times as long as broad. Single forms grow to

* Hueppe, *Mittheil a.d. Gesundheitsamt*, N. Band.

double their length, and then undergo division. They also form threads which may be composed of long rods, short rods, and cocci. They are motile, and provided with a flagellum at each end. If the nourishing medium is impoverished, the multiplication of the rods by division gradually ceases, and spore-formation commences. The rods become motionless, and a dark spot is visible, either in the middle or towards one end. This gradually develops into a shining spore with a dark contour. The rods swell slightly during this process, their contour becomes undefined, and soon disappears entirely, so that the spores are set free in about twenty-four hours. The spores are 1·2 μ long, and ·6 μ broad. They develop into rods in the following way. On one side of the spore a swelling appears, at the summit of which an opening in the spore-membrane results, and the germ escapes. This lengthens into a rod, and remains for a time attached to the empty spore-membrane. These spores are widely distributed, and occur in the air, soil, dust, etc. On the excrement of herbivorous animals the bacilli form a white efflorescence, and on infusion of horse-dung a thick crumpled skin. They flourish equally in liquids and upon damp, solid, nourishing media. On potatoes they grow as a yellowish-white skin ; on ordinary nutrient liquids they develop a thin, and subsequently a thick, dense, crumpled pellicle, with copious spore-formation. They are ærobic; deprivation of oxygen

causes the growth of the bacilli to cease, and the rods degenerate. They may be cultivated in various other nourishing media, such as blood-serum, nutrient gelatine, and nutrient agar-agar (Plate XIV., Fig. 2).

The simplest way to obtain a culture of the bacillus is to make a decoction of hay. The hay is chopped into small pieces, and boiled with distilled water in a flask for a quarter of an hour; it is then filtered into a beaker, which must be covered with a glass plate, and set aside in a warm place. In two or three days the liquid swarms with the bacilli, the spores of which exist in great numbers in ordinary hay. A more sure method for obtaining a pure cultivation is as follows:—

(*a*) Add only a small quantity of water to some finely chopped hay, and set aside for four hours at 36° C.

(*b*) Pour off the extract, and dilute it to the Sp. Gr. 1·004.

(*c*) Boil gently for one hour in a bulb plugged with cotton wool.

(*d*) Set aside 500 ccm. of the extract at 36° C.

In about twenty-four hours, as a rule, a pellicle has commenced to develop upon the surface of the liquid. If the reaction is definitely acid, carbonate of soda solution must be added to the decoction.

METHODS OF STAINING HAY BACILLUS.

To demonstrate the flagella of the bacilli, they may be stained with hæmatoxylin solution (Koch).

The linking together of cocci, long rods, and short rods in the threads, is shown by treating with alcoholic solution of fuchsine, or with iodine solution (Zopf).

To stain the spores the cover-glass preparations must be heated to a very high temperature (210° C.), in the hot-air steriliser for half an hour, or they may be exposed for a few seconds to the action of concentrated sulphuric acid (Büchner), or floated for twenty minutes on hot solution of the dye.

Bacillus ulna, Cohn.—Cocci, short rods, long rods, and threads. Diam. of the cocci 1·5—2·2 μ. Spore-formation in both short and long rods. No septic odour is produced by this bacillus in a nourishing liquid. Cloudy masses are found on the surface of the liquid, which later form a thick dry pellicle. The latter consists of bundles of threads matted together. The formation of ellipsoidal spores occurs in the usual way; they measure 2·5—2·8 μ long, and more than 1 μ wide. The bacillus is found in rotting eggs, and can be cultivated on boiled white of egg. It is closely allied to *Bacillus subtilis*.

Bacillus tumescens, Zopf.*—Cocci, long and short rods. They form a jelly-like disc, ·5—1 cm. in diam., on slices of boiled carrot, with the appearance of a rather tough crumpled skin of a whitish colour. Examination of this pellicle shows that it

* Zopf, *Die Spaltpilze*. 1885.

is formed of rows of rods lying closely together. These rods can be observed to divide into short rods and cocci. Spore-formation occurs in two stages of development, viz., in the cocci and in the short rods. A cultivation is obtained by exposing slices of boiled carrot, slightly moistened, to the air at the temperature of the room.

Bacillus megaterium, De Bary.—Large rods 2·5 μ wide, and four to six times as long. They

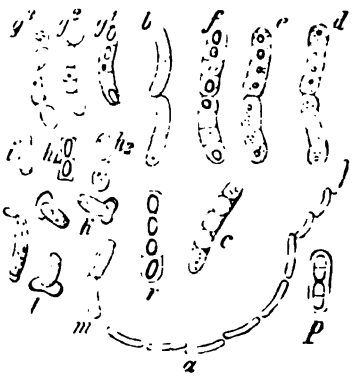


FIG. 120.—BACILLUS MEGATERIUM.
a. A chain of rods, × 250. The rest × 600.
b. Two active rods.
d to *f*. Successive stages of germination.
h and *l*. Successive stages of germination.
[After De Bary.]

are usually somewhat curved. Transverse division occurs, each segment attaining the length of the original rod. In the fresh state they appear non-articulated, but when treated with a dehydrating agent (tincture of iodine, alcohol), they are seen to be composed of short segments. The rods are motile, and form irregular chains, of a disjointed appear-

ance. They can be cultivated on nutrient agar-agar and nutrient gelatine. The latter is slowly liquefied, but the appearances are not characteristic. Spore-formation occurs in the usual way (Fig. 120). It was first observed on boiled cabbage.

Bacillus figurans (*Wurzel Bacillus*, *Bacillus mycoides*, Flügge).—Rods, with rounded ends, varying in length. Spore-formation present. In plate-cultivations they cause a cloudy growth, spreading from various points; if a cover-glass impression is made, this is found to consist of the regularly-arranged parallel rods. The chains of rods become twisted at intervals into curious convolutions, from which off-shoots are continued in various directions. These long shoots or processes are again twisted at intervals into varying shapes and patterns (Plate XXV., Figs. 1 and 2). Cultivated in nutrient gelatine, the bacilli form on the surface visible windings, from which fine filaments grow down into the gelatine. They spread out also in almost parallel lines transversely from the needle track. On an oblique surface of nutrient agar-agar the filaments spread downwards into the substance of the jelly, and

FIG. 121. PURE CULTIVATION OF BACILLUS FIGURANS IN NUTRIENT AGAR-AGAR.

outwards from the central streak on the surface, forming a feather-like cultivation* (Fig. 121). They are present in garden earth, and have also been cultivated from the air when charged with dust raised from the soil.

Bacillus tremulus.—Rods shorter and thinner than those of *Bacillus subtilis*. They are provided with a flagellum at both ends, and exhibit characteristic trembling and rotatory movements. Spores thicker than the bacillus, and often placed laterally. They were observed on rotting plant infusions, forming a thick slimy skin.

Bacillus of jequirity, Sattler.—Rods 2—4·5 μ long and ·58 μ thick. They can be cultivated on nutrient gelatine and blood serum. Infusion of jequirity containing the bacilli, or an artificial cultivation of the bacilli, inoculated into the conjunctiva of healthy rabbits produces severe ophthalmia. The poisonous principle is, however, believed to be a chemical ferment, *abrin*, and not the bacillus. Boiling, which does not destroy the spores of the bacillus, destroys the ferment, and cultivations started with these spores, though teeming with jequirity bacilli, are quite harmless.† The bacilli occur in infusions of the beans of *Abrus precatorius*, or jequirity.

Bacillus caucasicus, Kern.—Rods forming two spores, one at each end, otherwise similar to

* Described by the author, "Notes from a Bacteriol. Laboratory," *Lancet*. 1885.

† Klein, *Micro-organisms and Disease*. 1885.

Bacillus subtilis. They occur in the form of whitish lumps in company with *Saccharomyces mycoderma* in the production of a drink "kephir" from cow's milk. The fermentation is not due to the bacillus.

Bacillus dysodes, Zopf.—Cocci, long and short rods, and spores. They were observed in bread, making it greasy and unfit for food, and generating a penetrating odour resembling a mixture of peppermint and turpentine. A great loss may result to bakers if the fungus is introduced with the yeast.

Bacillus Hansenii, Rasmussen.—Rods 2·8—6 μ long, ·6—·8 μ wide. Cultivated on sterilised potato in four days they form a chrome-yellow layer with an agreeable fruitlike smell. Two or three days later the growth dries, and changes to orange-yellow in colour; later it passes to yellowish or brown, and forms at the same time spores 1·7 μ long, 1·1 μ wide. The colouring matter is insoluble in most reagents.

The bacilli occur on nourishing solutions, malt infusion, broth, wine, which have been kept at 31 to 33° C., as a yellow or whitish skin.

Bacillus erythrosporus, Cohn.—Motile rods and threads; rods exhibiting spore-formation. They grow well in nutrient gelatine, colouring the medium green by transmitted light. They were found to form a pellicle on meat-extract-solutions and on rotting albuminous liquids.

Bacillus septicus, Klein.*—Rods varying in size, non-motile. They form threads or leptothrix filaments, and are rounded at the ends. They are anærobic, and form spores independently of access of air. In a nourishing fluid they are overcome by the presence of *micrococci*, *Bacterium termo* or *Bacillus subtilis*. They occur in the soil, in putrid blood, and many putrid albuminous fluids, and occasionally in the blood-vessels of man and animals after death.

Bacillus saprogenes, Rosenbach.—Three rod-formed organisms have been described by Rosen-

FIG. 122.—BACILLUS SAPROGENES, No. 1. [After Rosenbach.]

bach as intimately associated with putrefactive processes.

No. 1.—Large rods (Fig. 122), which form an irregular sinuous streak with a mucilaginous appearance, when cultivated on nutrient agar-agar. Spore-formation present. They grow also very readily on blood serum, and all cultivations yield the odour of rotting kitchen refuse. They are not pathogenic.

No. 2.—Rods shorter and thinner than No. 1. They develop very rapidly on agar-agar, forming

* Klein, *Micro-organisms and Disease*. 1885.

transparent drops, which become grey. They were isolated from a patient suffering from profusely-sweating feet. The cultivations yield a characteristic odour similar to the last. They are pathogenic in rabbits. They appear to be identical with *Bacillus fœtidus* (*Bacterium fœtidum*, Thin).

No. 3.—See *Bacterium saprogenes.*

Bacillus fœtidus (*Bacterium fœtidum*, Thin).—Cocci, short rods, long rods, and leptothrix. The cocci, 1·25—1·4 in diam., occur singly or in pairs. Spore-formation present in the rods. They were isolated from the exudation in a case of profuse sweating of the feet, and the odour was noticeable in the cultivation (*vide Bacillus saprogenes*).

Bacillus putrificus coli, Bienstock.—Slender, motile rods, 3 μ in length, often less, sometimes forming long threads. Spore-formation present. Cultivations in gelatine are iridescent. They are constantly present in fæces.

Bacillus saprogenes fœtidus, Schottelius.—Rods with rounded ends, shorter, but about same width as the hay-bacillus. Immotile; spore-formation present. On nutrient gelatine the colonies are yellowish, and do not liquefy the medium. On potatoes they form a pale grey layer. They develop a strong rotting odour. They were isolated from the organs and intestinal contents of pigs reputed to be ill with swine-erysipelas.

Bacillus aerophilus, Liborius.—Slender rods, two-thirds the width of the hay-bacillus, and

thread-forms. Spore-formation present. In nutrient gelatine they form dot-like colonies of greenish-yellow colour, which liquefy the gelatine. In test-tubes a somewhat funnel-shaped liquefaction results. On potatoes they develop a yellowish layer. Powerfully ærobic. Found as a contamination.

Bacillus mesentericus fuscus, Flügge.—Small, short, actively-motile bacilli, often linked in twos and fours. Spore-formation present. They form white colonies on plate-cultivation, which later stream out in rays at the periphery, and liquefy the gelatine. In test-tube cultivations a funnel-shaped turbidity is produced, and then a stratum of liquefied gelatine with subsiding flocculi. On potatoes they develop a smooth yellowish layer, which soon becomes folded and wrinkled, forming a delicate veil over the nutrient surface. They occur on unsterilised potatoes.

Bacillus mesentericus vulgatus, Flügge (*Potato bacillus*).—Rods, longer and thicker than the above, and sometimes thread-forms; spore-formation present. The colonies, at first somewhat transparent, have later an opaque centre, and liquefy the gelatine. In test-tubes of nutrient gelatine a funnel-shaped turbidity results, and then an upper-stratum is completely liquefied, while a skin floats on the surface, and flocculent masses subside to the bottom of the liquid layer. They occur on potatoes.

Genus V.—Vibrio.

SPECIES.

UNASSOCIATED WITH DISEASE:—

Vibrio rugula . . . . Zymogenic saprophyte.

Vibrio rugula, Müller.—Rods and threads, 6—16 μ long, about ·5—2·5 thick. The rods are either simply bowed, or possessed of one shallow spiral (Fig. 123). They bear a flagellum at each

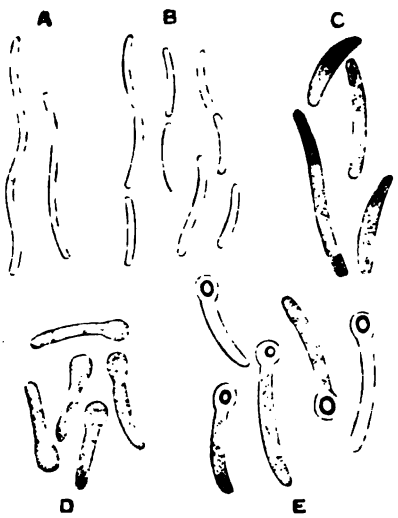


FIG. 123.—VIBRIO RUGULA, × 1020. A. Bowed threads. B. Slightly-curved rods. C. Rods swollen preparatory to spore-formation. D. Rods swollen at the spore-forming end. E. Various stages of the developing spores. [After Prazmowski.]

end. The rods form swarms when causing decomposition, and then, or after, grow out into threads, curved in a screw-like manner. In the next stage of development the rods cease to move, and become swollen with granular contents. One

extremity develops an enlargement, giving the rod the appearance of a pin. The spore formed by the contraction of the plasma in the swollen end finally becomes globular. The vibrios appear in vegetable infusions, causing fermentation of cellulose.

Genus VI.—Clostridium.

SPECIES.

ASSOCIATED WITH DISEASE IN ANIMALS :—		
	Clostridium of symptomatic anthrax	Pathogenic.
UNASSOCIATED WITH DISEASE :—		
	Clostridium butyricum . . .	Zymogenic saprophytes.
	Clostridium polymyxa . . .	,, ,,

Clostridium butyricum, Prazmowski (*Bacillus amylobacter*, Van Tieghem; *Bacillus butyricus. Bacillus of butyric acid fermentation*).—Rods 3—10 μ long, and under 1 μ wide, often indistinguishable from *Bacillus subtilis*. They grow out into long, apparently unjointed threads. They are mostly actively motile, but also occur in zooglœa. The rods and threads are sometimes slightly bent like vibrios. They are anærobic. The shorter rods as a rule swell in the middle, becoming ellipsoidal, lemon or spindle-shaped; the long rods, and sometimes the short ones, swell at one end; in either case ellipsoidal spores are developed (Fig. 124).

If they be cultivated in nutrient gelatine, the medium is liquefied, and a scum formed on the surface. They grow best between 35° and 40° C. The spores are widely distributed in nature, and

grow readily on fleshy roots, old cheese, etc. They convert the lactic acid in milk into butyric acid, and produce the ripening of cheese. They occur

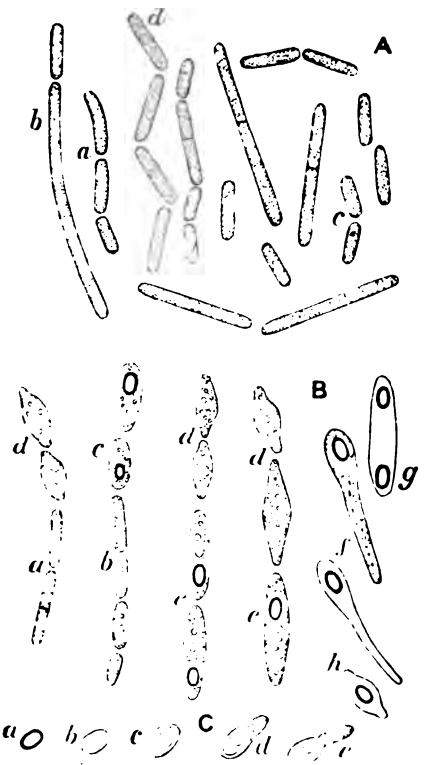


FIG. 124.—CLOSTRIDIUM BUTYRICUM.

A. Active stage. (*a*, *b*) Bent rods (vibrio-form) and threads. (*c*) Short rods. (*d*) Long rods.
B. Spore-formation. C. Spore-germination. [After Prazmowski.]

also in solutions of starch, dextrine, and sugar, and are the active agents in the fermentation of sauerkraut and sour gherkins.

METHOD OF STAINING THE BACILLUS OF BUTYRIC ACID FERMENTATION.

Treat the bacilli with iodine-solution. At certain stages of the fermentation-process the plasma takes a blue or violet-black coloration. The young rods give the former appearance, and the older ones the latter. It is most easily observed when the bacillus is cultivated in a substance containing starch, or, if starch is wanting, in the presence of cellulose, calcium-lactate, or glycerine; in bacilli cultivated in sugar solutions the reaction seldom appears.

Clostridium polymyxa, Prazmowski.—Threads consisting of rods which vary in length; cocci, involution-forms, and spores are also present; cultivated on nourishing solutions they develop a thick skin on the surface. On boiled beet and other roots they form a gelatinous scum, which often consists of crinkled, tough masses, several cm. in diam., somewhat similar to the *Ascococcus Billrothii*. They cause fermentation in solutions of dextrine, and more actively in potato or bean paste. Some cells give the iodine reaction weakly, as in *Clostridium butyricum*.

Clostridium of symptomatic anthrax (*Rauschbrand, Charbon symptomatique.**)—Rods rounded at the ends, mostly with a shining spore at one end. They are especially distinguished from the bacilli of anthrax by being motile. Cultivated on blood-

* Arloing, Cornevin et Thomas, *Bull. de l'Acad. de Méd.* 1881.

serum, threads develop, consisting of both rods and cocci. From blood-serum they can be cultivated on nutrient gelatine, and vegetable albumen.

Cultivation does not deprive the micro-organism of its virulence, but heating the spores to 85° C. renders them harmless.

Inoculation in the subcutaneous tissue of guinea-pigs, rabbits, calves, and sheep proves fatal. White rats, dogs, and fowls have an immunity. Injection into the veins in small quantity produces a febrile disorder, in larger quantities death. Animals in the former case suffer an abortive illness, which protects them against further inoculation. The micro-organism is the cause of a disease in cattle, "*blackleg*," "*quarter-evil*," or "*Rauschbrand*." At the autopsy the micro-organisms are found in the subcutaneous connective tissue, in the lymph glands, kidneys, spleen, and lungs. An irregular tumour is formed in the skin, which develops rapidly, and gives crepitus on palpation. The tumour, which is hæmorrhagic effusion, occurring in the extremities, impedes the animal's movements. The cattle infected die in thirty-six to forty-eight hours.

Group III. Leptotricheæ.

Genus I. *Crenothrix*.—Threads articulated; cells sulphurless; habitat water.

Genus II. *Beggiatoa*.—Threads unarticulated; cells with sulphur granules; habitat water.

Genus III. *Phragmidiothrix.*—Threads jointless; successive subdivision of cells is continuous; cells sulphurless; habitat water.

Genus IV. *Leptothrix.*—Threads articulated or unarticulated; successive subdivisions of cells not continuous; cells sulphurless.

Genus I.—Crenothrix.

SPECIES.

UNASSOCIATED WITH DISEASE:—

Crenothrix Kühniana . . Simple saprophyte.

Crenothrix Kühniana, Rabenhorst.—Cocci, rods, and thread-forms. The cocci are globular, 1—6 μ in diam. The threads are colourless, 1·5—5 μ thick, and club-shaped at the extremity, reaching a diam. of 6—9 μ. The threads form colonies with a brick-red, olive-green, or dark-brown to brown-black coloration caused by impregnation with oxide of iron. The threads are distinctly articulated, and ensheathed. The segments are set free when the sheath bursts, and develop into new threads. In other cases the segments remain enclosed, and subdivide into discs, which, by vertical fission, break up into globular forms (cocci). These again develop into new threads, either within the sheath eventually penetrating it, or after they are set free (Fig. 125).

The micro-organism appears in little whitish or brownish tufts in wells and drain-pipes, and it not only renders drinking-water foul, but may stop up the narrower pipes.

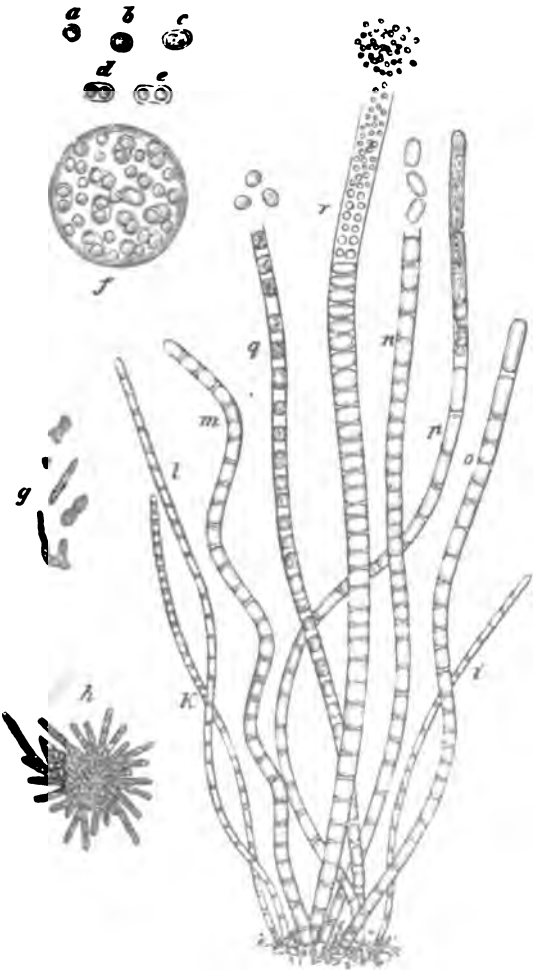


FIG. 125.—CRENOTHRIX KÜHNIANA.

a, *b*, *c*, *d*, *e*. Cocci in various stages of fission, × 600.

f. Zoogloea of cocci, × 600.

g. Various forms of zoogloea, natural size.

h. Colony of threads composed of rods grown out of a zoogloea of cocci.

i—*r*. Thread-forms; some straight, others spiral, with more or less differentiation between base and apex. (*r*) is composed of short rods at the base and above these of cylindrical segments, and at the apex these segments have divided into cocci, × 600. · [After Zopf.]

Genus II.—Beggiatoa.

SPECIES.

UNASSOCIATED WITH DISEASE :—	
Beggiatoa alba	Simple saprophyte.
Beggiatoa mirabilis . . .	„ „
Beggiatoa roseopersicina . .	Chromogenic saprophyte.

Beggiatoa alba, Vauch.—Cocci, rods, spirals, and threads (Fig. 126). The threads are longer and thicker than leptothrix, indistinctly articulated, actively oscillating, and colourless; their protoplasm contains numerous strongly refractive granules consisting of sulphur. They occur as greyish- or chalk-white gelatinous threads, 3—3·5 μ thick, in sulphur springs and marshes.

Beggiatoa mirabilis, Cohn.—Threads distinguished from others of this genus by their breadth, which may reach 30 μ. They are motile, bent and curled in various ways, and rounded at the ends. Around the threads isolated cells have been observed, "macrococci," but spiral forms are as yet unknown. The threads are filled with sulphur granules. They occur on sea water, forming a white gelatinous scum on decomposing algæ, etc.

METHODS OF EXAMINING SPECIES OF BEGGIATOA.

The articulation of the threads is best demonstrated by staining with an alcoholic solution of methyl-violet, fuchsine, or vesuvin; or by treating with sodic sulphate, or warm glycerine.

Beggiatoa roseo-persicina (*Cohnia roseo-persicina. Bacterium rubescens, or Peach-coloured bacterium*, Lankester).—Cocci, rods, spirals, and threads (Fig.

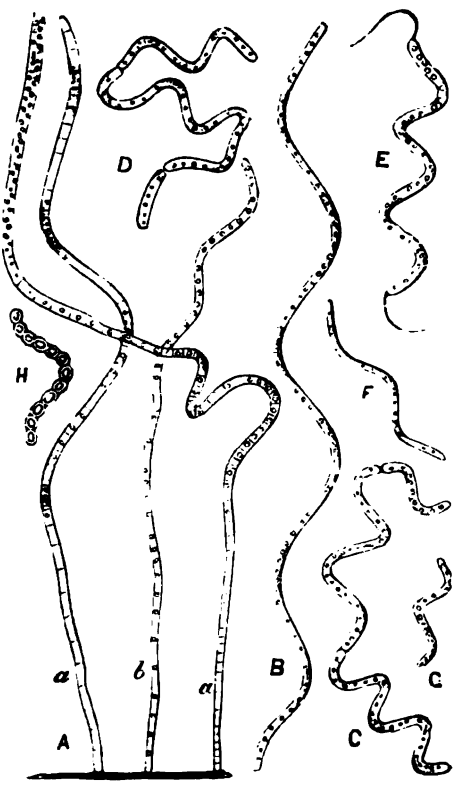


FIG. 126.—BEGGIATOA ALBA.

A. Threads, at base distinctly linked, partly spiral. B. A thread, spiral in its whole length. C, D. Fragments detached from threads; immotile. E. Active spirillum-forms, with a flagellum at either end. F. G. Thin and short spiral forms. H. A spiral showing the individual links. × 540. [After Zopf.]

127). The cocci, globular or oval, reach 2·5 μ in diam. They form at first solid families, bound to-

gether by gelatinous substance. Later they become larger, globular or ovoid in shape, and hollow, containing watery fluid in their interior. The families reach a diameter of 660 μ, in which the cocci form simply a peripheral layer. The hollow families or vesicles are often perforated, presenting a delicate reticulated appearance, which finally may become broken up into irregular structures. The red colour-

FIG. 127.—SEVERAL PHASE-FORMS OF BEGGIATOA ROSEO-PERSICINA. [After Warming.]

ing matter can be distinguished from other red pigments, and it is designated by the name bacteriopurpurin. It is quite distinct from the pigment produced by *Bacterium prodigiosum*, being peach-blossom red, and insoluble in water, alcohol, etc. Examined spectroscopically it shows a strong ab-

sorption in the yellow, and a weaker band in the green and blue, as well as a darkening in the more refrangible half of the spectrum. In the cocci, especially of the older vesicles, dark granules are to be seen, which consist of sulphur. The micro-organisms occur on the surface of marshes, or on water in which algæ are rotting. They form a rose-red, blood-red, violet-red, or violet-brown scum; and sometimes in such quantity that whole marshes and ponds may be coloured blood-red by them.

All the following, hitherto described as distinct species, are probably only phase-forms of Beggiatoa roseo-persicina.

Spirillum sanguineum, Cohn (*Ophidomonas sanguinea*).—Threads 3 μ and more in thickness with 2—2½ spirals, each 9—12 μ high, with their ends provided with flagella. Their colour is due to the presence of reddish granules contained in the cells. They were observed in brackish water with putrefying substances.

Spirillum rosaceum, Klein.—Resembles *Spirillum undula*, but is reddish in colour; the colouring matter is insoluble in water, alcohol, or chloroform.

Spirillum violaceum, Warming.—Threads, crescent-shaped, or possessing 1¼ or 1 spiral, with their ends broad, rounded, and provided with flagella. The colour is due to the contents, which are violet.

Monas vinosa.—Round or oval cells of about 2·5 μ in diameter, often united in pairs. Their motion is slow and tremulous, and the cell substance pale-red, with dark grains interspersed. Flagella have not been observed. They were observed in water with decaying vegetable matter.

Monas Okenii.—Short cylindrical cells, 5 μ wide, 8—15 μ long, with rounded ends. They undergo lively movements, each end being provided with a flagellum twice as long as the cell itself. They have pale-red cell-substance with dark grains. They occur in stagnant water.

Rhabdomonas rosea.—Spindle-form cells, 3·8—5·0 μ broad, 20—30 μ long. They exhibit slow, trembling movements, having at each end of the cell a flagellum. The cell substance is very pale, with dark grains interspersed. In brackish water.

Monas Warmingii.—Cylindrical cells, rounded at the ends; 15 μ long, 5—8 μ broad. They are possessed of a flagellum at each end of the cell, and exhibit rapid, irregular movements. The cell substance is pale-red, and studded at the rounded ends with dark-red grains.

Genus III.—Phragmidiothrix.

SPECIES.

UNASSOCIATED WITH DISEASE:—

Phragmidiothrix multiseptata . . Simple saprophyte.

Phragmidiothrix multiseptata.— Cocci and threads. The latter, 3—6 μ in breadth, are separated by transverse partitions into short cylindrical discs, whose height is a fourth or sixth of their breadth. Repeated transverse and longitudinal division takes place in the discs, resulting in the formation of cocci. The cocci have not been observed isolated from the threads in a free state, but they develop *in situ* into slender threads. In addition to this continuous subdivision, *Phragmidiothrix* differs from *Beggiatoa*

in the absence of sulphur, and from *Crenothrix* by its wanting a sheath. They occur attached to crabs (*Gammarus locusta*) in sea water.

Genus IV.—Leptothrix.

SPECIES.

ASSOCIATED WITH DISEASE:—

Leptothrix buccalis . . .	Saprophytic.
Leptothrix gigantea . . .	,,

Leptothrix buccalis, Robin. — Long, thin threads, ·7—1 μ broad, colourless, often united in thick bundles or felted together. Masses of cocci occur with the threads, and the threads themselves are composed of long rods, short rods, and cocci. The threads may break up into spiral-, vibrio-, and spirochæta-forms. The last-named occur in large numbers in the mouth, and have been named *Spirochæte buccalis*. The *Leptothrix buccalis* is found in teeth slime, and is believed to be intimately connected with dental caries. The threads penetrate the tissue of the teeth, after the enamel has been acted upon by acids generated by the fermentation of food. The short rods, long rods, cocci, leptothrix-forms, and screw-forms are found in the dental canals.

METHODS OF STAINING THE LEPTOTHRIX BUCCALIS.

The threads of *Leptothrix buccalis* have a special staining reaction (Leber). They become coloured if in an acid medium with iodine; if the medium is alkaline, it must be

acidified with very dilute hydrochloric acid or acetic acid, and the filaments then stained with iodine. The contents are stained violet, and contrast with the sheath and septa, which remain uncoloured.

Leptothrix gigantea, Miller. — Long rods, short rods, and cocci, can be observed in the same thread. There are also screw-threads, which may take the form of spirals, vibrios, or spirochætæ. The threads increase in diameter from base to apex, and corresponding with the thickness of the threads, the rods and cocci show different dimensions. They have been observed in the diseased teeth of dogs, sheep, cats, and other animals.

Group IV. Cladotricheæ.

Genus I.—Cladothrix.

Species.

Unassociated with Disease :—		
	Cladothrix dichotoma . . .	Saprophytic.
	Cladothrix Fœrsteri . . .	,,
Associated with Disease :—		
	Actinomyces	Pathogenic.

Cladothrix dichotoma, Cohn. — Threads resembling those of leptothrix; slender, colourless, not articulated, straight or slightly undulated, and in places twisted in irregular spirals with pseudo-branchings. The development can be traced from the cocci to rods and threads. The latter are at the beginning simple threads, which were

formerly described as *Leptothrix parasitica*, or if coloured by impregnation with iron, as *Leptothrix*

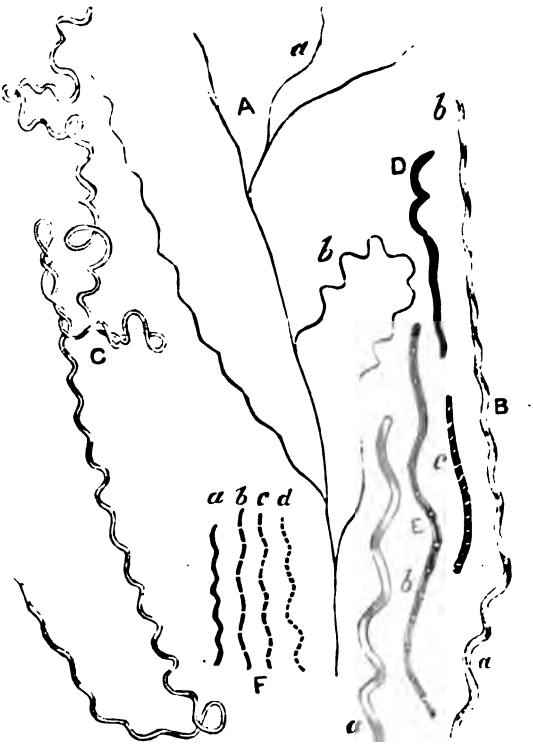


FIG. 128.—CLADOTHRIX DICHOTOMA.

A. Branching schizomycete : (*a*) Vibrio-form ; (*b*) Spirillum-form [slightly magnified].
B. A screw-form with (*a*) Spirillum-form ; (*b*) Vibrio-form.
C. Long spirochæta-form.
D. Fragment with spirillum-form at one end, vibrio-form at the other.
E. Screw-forms : (*a*) continuous ; (*b*) composed of rods ; (*c*) composed of cocci.
F. Spirochæta-form : (*a*) continuous ; (*b*) composed of long rods ; (*c*) short rods ; (*d*) cocci. [After Zopf.]

ochracea. Later they form false branches by single rods turning aside, which by repeated

division lengthen into threads. A thread appears to be first composed of long rods, then of short rods, and lastly of cocci. The iodine reaction must be applied to distinguish these forms, especially when the sheath of the threads has a yellow, rust-red, olive-green, or dark-brown coloration. The cocci may grow into rods while still in the sheath, and finally become leptothrix threads, surrounded by a delicate gelatinous sheath, from which the false branching proceeds. Fragments may break off, which are actively motile, and appear as vibrios, spirilla, and spirochæta-forms (Fig. 128). They may also occur in zooglœa.

They are the commonest of all bacteria in both still and running water, in which organic substances are present. They are observed also in the waste water of certain manufactures, such as sugar. Artificially they can be cultivated on infusions of rotting algæ and animal substances, forming on these media small tufts, about 1—3 μ, and floating masses.

Cladothrix Fœrsteri (*Streptothrix Förster*i, Cohn).—Cocci, rod-forms, and leptothrix-threads. The threads are twisted in irregular spirals, and branch sparingly and irregularly. Screw-forms are produced by the threads breaking up into small pieces. They occur in the lachrymal canals of the human eye, in the form of closely felted masses.

Here we may add some little-known species, which possibly belong to this group.

Sphærotilus natans.—Cells 4—9 μ long, 3 μ thick, united in a gelatinous sheath to form threads. The cells comprise rods and cocci-forms; the cocci are set free, and develop into rods, which again form threads. In the last a false branching has been observed. The plasma of the cells breaks up into minute, strongly refractive portions, which develop into round spores, at first of a red, and afterwards a brown colour. They occur in stagnant and flowing water contaminated with organic matter, and form floating flakes of a white, yellow, rust-red, or a yellow-brown colour.

Myconostoc gregarium, Cohn.—The threads are very thin, colourless, unarticulated, but fall apart into short cylindrical links when dried. They form gelatinous masses, 10—17 μ in diameter, singly or heaped into slimy drops on water in which algæ are decomposing.

Spiromonas volubilis, Perty.—Colourless, transparent cells, 15—18 μ long. Rapidly motile and revolving round a longitudinal axis. They occur in marsh water and putrefying infusions.

Spiromonas Cohnii.—Colourless cells, consisting of $1\frac{1}{4}$ spirals, with both ends acutely pointed and provided with a flagellum. Breadth of the cells 1·2—4 μ. They occur in water containing decomposing matter.

The following species is described last, so that it may stand between the cladothrix group of bacteria and the hyphomycetous fungi given in the appendix which follows. It is attached provisionally to the former for reasons stated below, but there is need for further investigation before its position is established, as there is still some doubt as to the true life-history of the fungus.

There is also reason for believing that there are different forms of actinomycosis in animals, and that the rare disease in man differs etiologically from the not so uncommon disease in cattle. It is only then provisionally that the different forms are here described under one heading.

Actinomyces. — Actinomycosis is a disease occurring in animals * and occasionally in man.† It is caused by a parasite known as *Actinomyces*, or the "ray-fungus." The parasite appears in the form of a rosette of pyriform or club-shaped elements (Plate XXIX., Fig. 1). The little masses are colourless, pure white, or of a yellowish or yellowish-green tinge, and visible to the naked eye.

The fungus is believed to effect an entrance to the animal by the mouth, being taken in with the food, possibly through the medium of a wound of the gum or a carious tooth. In whatever manner it has gained access to the living organism, it sets up inflammation in its neighbourhood, resulting in the formation of a neoplasm, composed chiefly of round cells, resembling a tuberculous nodule. The nodules may break down and suppurate, or may go on increasing in size. Fibrous tissue develops between the nodules, and large tumours eventually result containing purulent cavities and excavations.

* Bollinger, *Centralbl. f. Med. Wiss.* 1877.

† Israel, *Virchow's Archiv*, vols. 74—78. Pontick, *Die Actinomycose des Menschen.* 1882; and *Beitr. z. Kenntn. der Actinomycose des Menschen.* 1882; *Lancet*, May 2nd, 1885; Conti, *Gaz. Med. Ital. Lombordia.* 1885; Acland, *Trans. Path. Soc.* 1886.

In the slimy detritus the little pale-yellow grains of fungus can be detected. In cattle the lower jaw is usually affected, and then the upper jaw and neighbouring parts. The organism may also occur in nodular tumours in the lung, subcutaneous and intermuscular tissues. It is the cause of "wooden tongue," and also of diseases which have been variously described before their true nature was understood as bone-canker, bone-tubercle, osteo-sarcoma, etc.

In man the pulmonary formations tend to break down early, forming fistulæ and sinuses, with the clinical character of empyema. In one case there were the symptoms of chronic bronchitis with fœtid expectoration. In other cases the disease, originating in the lung, spread to the prævertebral tissues. If the actinomyces invade bones, as has been especially observed in the bodies of the vertebræ, caries results. In another group of cases the organism has been said to produce disease of the intestinal canal. The fungus has also been detected in the crypts of the tonsils of healthy pigs, and a similar, if not identical, one in the spermatic duct of the horse.*

The disease has been transmitted from cattle to cattle by inoculation,† and a rabbit has been infected by means of a piece of actinomycitic tumour

* Johne, *Bericht über das Veterinärwesen im Königreich Sachsen für das Jahr.* 1884.

† Johne, *Deutsche Zeitschr. f. Thiermedicin.* 1881.

from a human subject, introduced into the peritoneal cavity.

Until quite recently actinomyces has been classed as a *hyphomycete*, and the flask-shaped structures regarded as gonidia. By certain* cultivation-experiments we are led to regard the latter as a result of a degenerative stage in the life-history of the fungus accompanied by the development of involution-forms. Inoculations of nutrient gelatine, in the form of plate-cultivations, and inoculations on blood serum and nutrient agar-agar were made, it is stated, with success. The cultures developed on the latter in from five to six days, growing best at a temperature of 33°—37° C. Nutrient gelatine was not liquefied. The appearances of the cultivation were described as quite characteristic; it has at first a whitish, granular appearance, followed after a few days by little yellowish-red spots which coalesce in the centre, and finally a whitish downy layer results with a golden-red centre; in time the periphery also becomes dotted with little yellow-centred masses. The fungus thus cultivated has been described as corresponding on examination with the form found in man and animals, and at one stage to consist of thread-forms, short rods, and cocci. As a result of these observations actinomyces has been relegated to the bacteria, forming one of the cla-

* Boström, "Ueber Actinomycose," *Verhandlungen des Congresses für Inn. Med.* 1885.

dothrix group, and possibly closely allied to the *Streptothrix Fœrsteri* of Cohn.

METHODS OF EXAMINING AND STAINING ACTINOMYCES

In the fresh state a little of the tissue of a tumour, or the purulent detritus, may be transferred to a clean glass slide, and teased out with needles. The little specks are easily recognised, and can be isolated with the needles and transferred to a drop of glycerine upon a fresh slide. A cover-glass must then be gently pressed down upon the preparation, which is then examined. To stain the fresh tufts, the little fungus masses are teased out, and transferred to a watch-glass containing alcohol, to which a few drops of concentrated alcoholic solution of eosin are added. They can be mounted and preserved in glycerine.

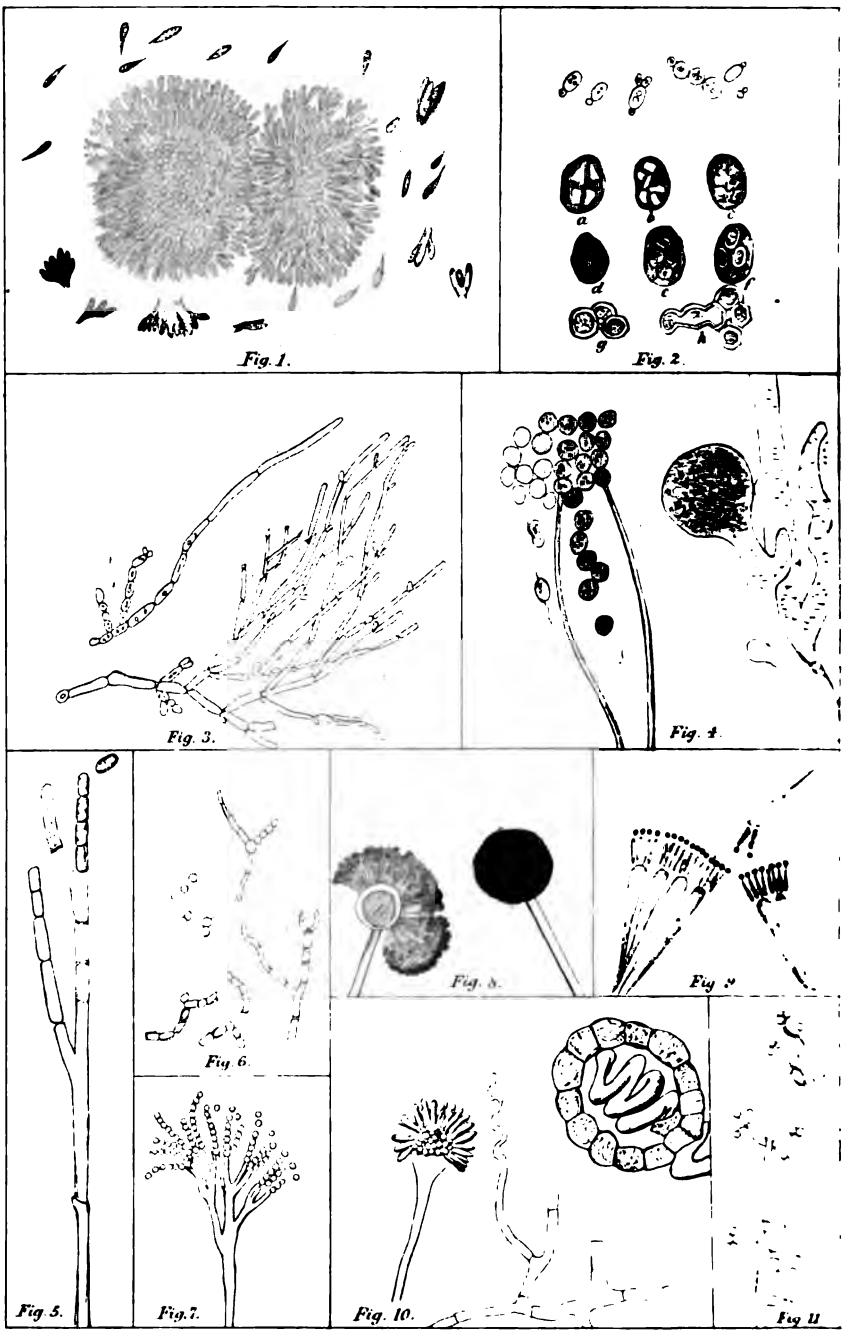
Sections can be stained by either of the following methods.

Weigert's Method:—

Immerse the sections for one hour in orseille, rinse with alcohol, and after-stain with gentian violet (Plate XXVIII.).

Plaut's Method:—

Sections are left for ten minutes in Gibbes' solution (No. 22), warmed to 45° C. They are then rinsed in water, and after-stained in concentrated alcoholic solution of picric acid for five to ten minutes; immersed in water five minutes, 50 per cent. alcohol fifteen minutes, passed through absolute alcohol and clove-oil, and preserved in Canada balsam (Plate XXVII.).



YEAST-FUNGI OR SACCHAROMYCETES AND MOULD-FUNGI OR HYPHOMYCETES.

DESCRIPTION OF PLATE XXIX.

FIG.

1.—*Actinomyces*, teased out in the fresh state and stained with eosin.

2.—*Torula cerevisiæ* (after Rees).

3.—*Saccharomyces mycoderma*, or *oidium albicans*, from an artificial cultivation (after Grawitz).

4.—*Saprolegnia* (after Sachs).

5.—*Oidium lactis* (after Flügge).

6.—Fungi of *favus*, or *oidium lactis* (after Neumann).

7.—*Penicillium glaucum* (after Flügge).

8.—*Aspergillus niger*, from a preparation mounted in glycerine.

9.—*Aspergillus niger*, from the same preparation (Zeiss $\frac{1}{12}$ o. 1).

10.—*Aspergillus glaucus* (after De Bary).

11.—*Botrytis Bassiana* (after De Bary).

APPENDIX A.

YEASTS AND MOULDS.

Yeast-fungi and *mould-fungi*, like *bacteria* or *fission-fungi*, are *achlorophyllous Thallophytes*. They belong to two separate orders, the *Saccharomycetes* and *Hyphomycetes*, which are intimately related to each other, but quite distinct from *bacteria*. Their germs occur widely distributed in air, soil, and water, and are constantly encountered in bacteriological investigations. In addition many species are of hygienic and pathological interest or importance in being either accidentally associated with, or actually the cause of various morbid processes. For a complete account of all the described species and full details of the various forms of development,* reference must be made to botanical treatises. A description of certain species is appended here, and may afford some useful information to the worker in a bacteriological laboratory.

YEAST-FUNGI OR SACCHAROMYCETES.

Saccharomyces cerevisiæ (*Torula cerevisiæ*).—Cells round or oval, 8—9 μ long, singly or united in small chains. Spores occur three or four together in a mother-cell, 4—5 μ in diameter (Plate XXIX., Fig. 2).

Sacch. ellipsoideus.—Elliptical cells, mostly 6 μ long, singly or united in little branching chains. Two to

* Sachs, *Text-book of Botany*. 1882.

four spores found in a mother-cell, 3—3·5 μ in diam. It is widely distributed, and is the principal agent in accidental fermentation.

Sacch. conglomeratus.—Cells round, united in clusters, consisting of numerous cells produced by budding from one or a few mother-cells. There are 2 to 4 spores in each mother-cell. They occur on rotting grapes and in wine at the commencement of fermentation.

Sacch. exiguus.—Conical or top-shaped cells, 5 μ long, and reaching 2·5 μ in thickness, in slightly branching colonies. Spore-forming cells are isolated, each containing 2 or 3 spores in a row. Present in the after-fermentation of beer.

Sacch. pastorianus.—Cells oval or club-shaped. Colonies consist of primary club-shaped links, 18—22 μ long, which build lateral, secondary round or oval daughter-cells, 5—6 μ long. Spores 2 to 4. In the after-fermentation of wine, fruit-wines, or fermenting beer.

Sacch. apiculatus.—Cells lemon-shaped, both ends bluntly pointed, 6—8 μ long, 2 — 3 μ wide. Budding occurs only at the pointed ends. Rarely united in colonies. Spores unknown. They occur with other yeasts in various accidental fermentations.

Sacch. sphæricus.—Cells varying in form; the basal ones of a colony oblong or cylindrical, 10—15 μ long, 5 μ thick; the others round, 5—6 μ in diam. United in ramified families. Spores unknown.

Sacch. mycoderma (*Mycoderma cerevisiæ et vini*). —Cells oval, elliptical, or cylindrical, 6—7 μ long, 2—3 μ thick, united in richly-branching chains. Spore-forming cells reaching 20 μ long. Spores 1 to 4 in each mother-cell. Forms the so-called "mouid" on fermented liquids, and develops on the surface without exciting fermentation. When forced to grow submerged, a little alcohol is produced, but the fungus soon dies. They occur on wine, beer, fruit-juices, and sauerkraut.

Sacch. albicans (*Oidium albicans*).—Cells partly

round, partly oval or cylindrical, 3 5—5 μ thick, the cylindrical cells 10—20 times as long as they are thick. The bud-colonies mostly consist of rows of cylindrical cells, from the ends of which oval or round cells shoot out. Spores form singly in roundish cells. They occur on the mucous membrane of the mouth, especially of infants, in greyish-white patches which consist of epithelium, bacteria, yeasts, and the mycelia of various moulds. They can be easily cultivated in a nutrient solution containing sugar and ammonic tartrate.* The cells germinate according to the richness of the fluid in sugar ; they either grow into long threads, or, in a very strongly saccharine solution, many daughter-cells are formed, budding out in various directions (Plate XXIX., Fig. 3).

Sacch. glutinis.—Cells round, oval, or short cylinders, 5—11 μ long, 4 μ wide, isolated, or united in twos. Cell-membrane and contents are colourless in the fresh state, but when dried and remoistened possess a pale-reddish nucleus in the middle. Spore formation unknown. Forms rose-coloured, slimy spots on starch, paste, or on sterilised potatoes. The colouring matter is not changed by acids or alkalies.

Sacch. rosaceus (*Pink Torula*).—Cells 9—10 μ in diam. Forms a coral-pink growth in nutrient gelatine, nutrient agar-agar (Plate XIII., Fig. 3), or on sterilised potatoes (Plate X., Fig. 2). They are present in the air.

Sacch. niger (*Black Torula*).—Cells also present in the air. Cultivated in nutrient gelatine they form a black crust (Plate III., Fig. 3).

MOULD-FUNGI OR HYPHOMYCETES.

The mould-fungi have been divided into five orders :† *Hypodermii*, *Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, and

* Grawitz, *Virch. Archiv*, vol. 70.

† Flügge, *Fermente u. Mikroparasiten.* 1883.

Myxomycetes. The following species, with the orders to which they belong, are of especial interest.

HYPODERMII.

Ustilago carbo (mildew, smut).—Spores, brown, circular; episporium smooth; sporidia, ovoid cells. The spores or conidia occur as a black powder in the ears and panicles of wheat, barley, and oats.

Tilletia caries.—Spores round, pale, brown; episporium with reticulated thickenings. In germinating sporidia grow out radially from the end of the promycelium; these, at their lower part, conjugate by a cross branch, and separate from the promycelium, and at some point of the pair, a hypha grows out on which abundant secondary sporidia develop. The latter are long, oval cells, which can in turn germinate. The fungus occurs in the form of a stinking powder in grains of wheat, which renders the meal impure, and gives it a disagreeable smell.

Urocystis occulta.—The spores consist of several cells united together; partly large, dark-brown cells in the interior, and outside several flat semi-circular, colourless cells. Spores ·024 mm. Promycelium germinates as in *Tilletia*, but the cylindrical cells produce a hypha, without, as a rule, previous conjugation. Occurs as a black powder in rye straw, in long disintegrated stripes, which are at first greyish. The affected plant produces abortive ears.

Empusa muscæ.—Spores, ·011 mm. in diam. A spore or conidium alighting upon the white area of the under surface of the body of the house-fly, germinates into a hypha. The latter, penetrating the skin, forms toruloid cells, which multiply by germination, and are disseminated in the blood throughout the body of the fly. These cells again grow into hyphæ, which penetrate the skin, each forming a conidium, which is cast off with considerable force. The parasite is fatal to flies, especially in the

autumn. They are often observed attached to the walls or window-panes, surrounded by a powdery substance, consisting of the extruded conidia.

Empusa radicans.—The spores form long hyphæ, which pierce the transparent skin of the caterpillar of the cabbage-white butterfly. The terminal cells ramify, and fill the body of the caterpillar with a network of mycelial filaments. The caterpillars attacked become restless, then motionless, and death ensues.

Tarichium megaspermum.—The spores are ·05 mm. in diam., black in colour, and provided with a thickened episporium. They occur at the sides and ends of mycelial threads, attacking caterpillars (*Agrotis segetum*).

PHYCOMYCETES.

Saprolegnia.—Colourless threads, forming dense radiating tufts, occur on living and dead animal and vegetable matter in fresh water. The filaments penetrate into the substratum, and branch more or less in the surrounding water. The cylindrical ends of threads are shut off by a septum forming zoosporangia, or mother-cells, in the interior of which a number of spherical spores, zoospores, develop. These are set free through an apical opening in the thread, and, after a time coming to rest, give rise to new plants (Plate XXIX., Fig. 4). In the sexual mode of reproduction, a spherical bud, the *oogonium*, develops at the end of a mycelial thread; from the thread, small processes or *antheridia* sprout out laterally towards the oogonium, and blend with its protoplasm (Plate XXIX., Fig. 4). The latter breaks up into a number of *oospores*, which clothe themselves with a membrane, while still within the mother-cell, and eventually being set free, grow into fresh mycelial filaments. The parasite attacks fish and tritons, and produces a diseased condition of the skin, which may be ultimately fatal. In salmon it produces the common disease of salmon.

Peronospora infestans.—Mycelium, ·005 mm. in

thickness. Twigs with as many as five branches, each bearing an egg-shaped conidium. The contents of the conidia falling off and reaching a drop of moisture, break up into a number of swarming zoogonidia, which in turn develop upon plants. Fixing themselves to the cuticle of the host, they throw a germinating filament into an epidermal cell; piercing first its outer wall, and then its inner wall, the filament reaches an intercellular space, where the mycelium develops. This continues to grow and spread throughout the plant. In tubers it can hibernate and develop in the young shoots in the following spring. The parasites appear in the form of brown patches on the green parts of the plants, especially the leaves. The attacked parts wither and turn yellow or brown in colour. If the under surface of a diseased leaf be examined, a corresponding dark spot may be observed, accompanied with a faint greyish-white bloom which covers it. The latter consists of the conidia-bearing branches of the fungus.

Pilobolus.—Hyphæ, 1—2 mm. high. Fruit-hyphæ, possessing spherical receptacles containing conidia. When ripe the receptacles with their conidia are detached at their bases and spring by their elasticity to some distance. The mould occurs as glassy tufts on the excrement of cows, horses, etc. A cultivation can generally be obtained by keeping fresh horse-dung under a bell-glass.

Mucor mucedo.—Hyphæ, colourless, simple or branched, 1—15 cm. long, sporangia are yellowish-brown or black. Spores ovoid, ·008 mm. long, and ·0037 wide. Occurring as the familiar white mould on fruits, bread, potatoes, excreta, and penetrating into the interior of nuts and apples. A network of fibrils develops in the substance of nutrient gelatine, with formation of sporangia on the free surface. The germination of the spores and development into hyphæ can be observed in a few hours, if the fungus be cultivated in a decoction of horse dung.

Mucor racemosus.—Hyphæ, at most 1·5 cm. long

sporangia, yellowish to pale-brown; spores round. By continued cultivation in liquids saturated with carbonic acid, the hypha becomes shorter, and exhibits a yeast-like sprouting. These yeast-like or toruloid cells can, when the carbonic acid is withdrawn, germinate into normal mycelium.

Mucor stolonifer, Lichtheim.—Mycelium grows in the air, and then bends down and re-enters the nutrient substratum; sporangia black, and spores globular. The mycelium can penetrate through the shell of eggs, and form conidiophores within them.

Mucor aspergillus, Lichtheim. Fruit hyphæ, thinned at the base, and with many fork-like divisions, dark-brown spores.

Mucor phycomyces, Lichtheim.—Mycelium thick-walled, olive-green fruit-hyphæ, black sporangia, and oblong spores.

Mucor macrocarpus, Lichtheim.—Spindle-formed, pointed spores.

Mucor fusiger, Lichtheim.—Ovoid spores.

Mucor mellittophorus, Lichtheim.—Spores elliptical. Found in the stomach of bees.

Mucor corymbifer, Lichtheim.—Forms branched fruit-hyphæ; sporangium has a smooth membrane. Found in the external auditory meatus; occurring also upon bread. Pathogenic in rabbits.

Mucor rhizopodiformis, Lichtheim.—Occurs on bread. The spores of *Mucor rhizopodiformis* and *Mucor corymbifer*, when introduced into the vascular system of rabbits, can germinate in the tissues, especially in the kidneys, where they set up hæmorrhagic inflammation. Dogs are immune, and only artificial mycosis is known.*

* Lichtheim, *Zeistchr. f. Klin. Med.*, vii.; Hückel, *Beitr. z. Anat. u. Phys.*, herausgeg. v. Ziegler u. Nauwerck. 1885.

ASCOMYCETES.

Oidium Tuckeri.—Fruit hyphæ, bearing single ovoid conidia. Observed in the form of brown patches, covered with a white mildew-like layer on the leaves, branches, and young fruit of the vine, producing a "grape-disease."

Oidium lactis.—Fruit hyphæ, simple, erect, and colourless, bearing at their ends a series or chain of conidia (Plate XXIX., Fig. 5). In some cases the fruit hypha branches beneath the chain of spores. Spores are short cylinders, ·0077—·0108 mm. long. The fungus is sometimes found as a whitish mould on milk, bread, paste, potato, and excrement, and is believed to be identical* with the fungus of certain human skin diseases, *Favus* (*Achorion Schoenleinii*), *Herpes tonsurans* (*Tricophyton tonsurans*) and *Pityriasis versicolor* (*Microsporon furfur*) (Plate XXIX., Fig. 6). Cultivated artificially in nutrient gelatine, the conidia germinate into filaments of varying length, which by subdivision form septate mycelial hyphæ; these and their branches give rise in turn to spores or conidia. The differences observed in various diseases are attributed to differences in the nutrient substratum. Others† maintain that, in artificial cultivations of the spores of *Tricophyton tonsurans*, the fructification is identical with Penicillium.

Oidium albicans.—Vide *Saccharomyces albicans.*

Aspergillus glaucus (*Eurotium aspergillus glaucus*).—Mycelium, at first whitish, becoming grey-green or yellow-green. Spores grey-green, thick-walled, ·009—·015 mm. in diam. Sometimes found on various substances, chiefly cooked fruit (Plate XXIX., Fig. 10).

Aspergillus repens (*Eurotium repens*), De Bary.—Fruit heads fewer than in the above, which are at first pale and then blue-green to dark-green in colour; conidia mostly oval, smooth, ·005—·008 mm. long, colourless or pale to grey-green.

* Grawitz, *Virchow's Archiv*, vol. 70.

† Morris and Henderson, *Journ. Royal Microsc. Society.* 1882.

Aspergillus flavus.—Gold-yellow, greenish and brown tufts; fruit heads round; yellow, olive-green, or brown. Conidia round, seldom oval; sulphur-yellow to brown in colour, ·005—·007 mm. in diam. Saprophytic in man, pathogenic in rabbits.

Aspergillus fumigatus.—Greenish, bluish, or grey tufts. Fruit heads long and conical. Conidia round, seldom oval, smooth, mostly pale and colourless. Diam. ·0025 to ·003 mm. Observed saprophytically in human lungs, external auditory meatus, and middle ear. The spores introduced into the vascular system of rabbits, or into the peritoneal cavity, establish metastatic foci in the kidneys, liver, intestines, lungs, muscles, and sometimes in the spleen, bones, lymphatic glands, nervous system, and skin.

Aspergillus niger (*Eurotium aspergillus niger*, De Bary).—Dark chocolate-brown tufts. Conidia round, black-brown, or grey-brown, when ripe; ·0035 to ·005 mm. This mould can be cultivated readily on bread moistened with vinegar, on slices of lemon, and on acid fruits and liquids. It flourishes best of all, according to Raulin,* in a liquid of the following composition:—

	Grammes.
Water	1500
Sugar-candy	70·
Tartaric acid	4·
Nitrate of ammonia	4
Phosphate	·6
Carbonate of potassium	·6
„ „ magnesium	·4
Sulphate of ammonia	·25
„ „ zinc	·07
„ „ iron	·07
Silicate of potassium	·07

It was also found that the fungus grew best when the liquid was spread out in a layer 2 or 3 cm. in depth in a

* Duclaux, *Health Exhibition Handbook*, London, 1884.

shallow dish, and a temperature of 35° C. proved to be the most favourable. The abstraction of zinc from the nutritive liquid reduced the weight of a crop from 25 (the average) to 2 grammes, and the presence of $\frac{1}{100000}$ part of nitrate of silver, or $\frac{1}{50000}$ part of corrosive sublimate, stopped the growth altogether. It is saprophytic in the living body.

METHOD OF EXAMINING ASPERGILLUS NIGER.

Species of aspergillus stain intensely with carmine, fuchsine, or methyl-violet, but to examine *Aspergillus niger* with a high-power, a little special technique is employed, as follows:—A drop of glycerine is placed on a clean slide, and a drop of alcohol on a cover-glass. With a fine pair of forceps a few of the fruit hyphæ with their black heads are immersed in the alcohol. The cover-glass is then turned over on to the drop of glycerine, and the slide held in the flame of a Bunsen burner till the spores or conidia are dispersed. To make a permanent preparation, remove the cover-glass, and transfer the fruit hyphæ so treated to a mixture of glycerine and water (1 to 5); a drop may be conveniently placed ready on a slide provided with a ring of Canada balsam. The specimen is then permanently mounted by employing a circular cover-glass, and surrounding it with a ring of cement in the usual way (Plate XXIX., Figs. 8 and 9).

Aspergillus ochraceus.—At first flesh-coloured, and then ochre-yellow heads.

Aspergillus albus.—Pure white fruit heads.

Aspergillus clavatus.—Club-shaped fruit heads on long stems.

Penicillium glaucum.—Occurs as a white, and later a blue-green mould, on which dewlike drops of liquid may appear (Plate IX., Fig. 2). Its spores are present in large numbers in the air, and are liable to contaminate cultivations. Diam. of the spores ·0035 mm.; threads vary in diameter between ·004 and ·00071 mm.,

according to the nourishing material; the fruit hypha bears terminally a number of branched cylindrical cells, from which chains of greenish conidia are developed (Plate XXIX., Fig. 7). It is the commonest of all moulds.

Botrytis Bassiana.—Hyphæ and spores colourless. Hyphæ usually simple, but sometimes united in arborescent stems (Plate XXIX., Fig. 11). It is the cause of muscardine, a fatal disease of silkworms, and occurs also in various other caterpillars and insects.

UNCLASSED.

Chionyphe Carteri.—Mycelium, penetrating the skin and subcutaneous tissue, sets up suppuration and ulceration. Described as the cause of a disease known in India as "madura-foot."

APPENDIX B.

FLAGELLATED PROTOZOA IN THE BLOOD.*

WHEN examining blood the bacteriologist must be prepared to meet with minute organisms which at the first glance under moderate amplification may be mistaken for vibrionic or spiral forms of bacteria. The organisms referred to belong not to the vegetable, but to the animal kingdom. They may occur associated with disease, but they appear to be more commonly found in the blood of apparently perfectly healthy animals.

Flagellated organisms in the blood of rats and hamsters.—Lewis† described peculiar organisms in the blood of healthy rats in India. When first noticed

* Abstract of paper by the Author, *Journ. Roy. Micros. Soc.*, read November 10th, 1886. See also papers on the Micro-parasites of Malaria by Laveran, Marchiafava and Celli.

† Lewis, *Microscopic Organisms in the Blood of Man and Animals.* Calcutta, 1879 (with photographs); and *Quart. Journ. Micr. Sci.*, lxxiii. (1879), pp. 109-14, and xxiv. (1884), pp. 357-69.

they were thought to be vibrios or spirilla. A drop of blood under examination appeared to quiver with life, and on diluting the blood, motile filaments could be seen rushing through the serum, and tossing the blood-corpuscles about in all directions. Under careful examination the filaments were found to consist of a thicker portion or body, with at one end a flagellum (Fig. 129).

After fixing with osmic acid they measured 0·8—1 μ in width, and 20—30 μ in length ; the flagellum was about as long as the body, so that the total length of the organism was about 50 μ. Lewis detected these parasites in 29 per cent. of the species *Mus decumanus* and *Mus*

FIG. 129.—PARASITES IN THE BLOOD OF RATS [after Lewis].

rufescens. Though they had many features in common with motile organisms of vegetable origin, they appeared to approach much more closely to the Protozoa, more particularly several of the species of Dujardin's *Cercomonas*.

Wittich* discovered, in the blood of hamsters, whip-like bodies with lively movements. They resembled frog's spermatozoa, possessing a thick portion continued into a long lash-like thread. Wittich considered them identical with the organisms described by Lewis, and they also were observed in apparently healthy animals. Koch† later met with the same organisms.

* "Spirillen im Blut von Hamstern," *Centralbl. f. Med. Wiss.* 1881, No. 4.

† *Mittheilungen aus dem Kaiserlich. Gesundh. Amt.* 1881. 8.

Flagellated organisms in the blood of horses, mules, and camels.—In India a fatal disease, known by the natives as *Surra*, occurs in horses, mules, and camels. The malady is described as a blood disease, characterised by fever, accompanied by jaundice, petechiæ of mucous membranes, great prostration, and rapid wasting, terminating in death. Evans* observed the presence of a parasite in the blood, and by means of subcutaneous inoculation, and by the introduction into the stomach of blood containing the parasites, the disease was transmitted to healthy animals.

Steel,† who was deputed to investigate this disease in British Burma, also found the parasite in all cases, and further observed that it appeared as the temperature rose and disappeared during the apyrexial periods. This observer concluded that the organism was a spiral bacterium, and named it, after its discoverer, *Spirochæta Evansi.*

Flagellated organisms in the blood of fish.—In the blood of mud-fish (*Cobitis fossilis*) Mitrophanow‡ observed the presence of peculiar micro-parasites (Fig. 130). As a 1 per cent. salt solution had been added to the blood under examination, it occurred to Mitrophanow that they were possibly the cytozoa described by Gaule; but this idea was dismissed by the fact that they were found in blood to which no salt solution was added. Their size varied from 30 to 40 μ in length, and 1 to 1·5 μ in width. At first their rapid movements baffled examination, but as the rapidity lessened there was the appearance of a curling movement in the body portion, and a swinging movement of the lash. The organism moved in the direction of the lash, the anterior end of the body being

* Evans, *Report published by the Punjab Government Military Department*, No. 439. 1880.

† Steel, A.V.D., *An Investigation into an Obscure and Fata Disease among Transport Mules in British Burma.* 1885.

‡ "Beiträge zur Kenntniss der Hämatozoen," *Biol. Centrabl.*, iii., 1883, pp. 35-44.

more pointed than the posterior, and gradually fining off into the lash. When the body seemed to rest, the lash might be seen to whip out in all directions. As the movement of the body gradually diminished, it appeared to have a complicated screw-form, the axis of the screw corresponding to the body, to which an undulating membrane is fastened spirally. This could be distinguished when the organism was dying, because the body in death contracted, and the membrane then looked like a spiral

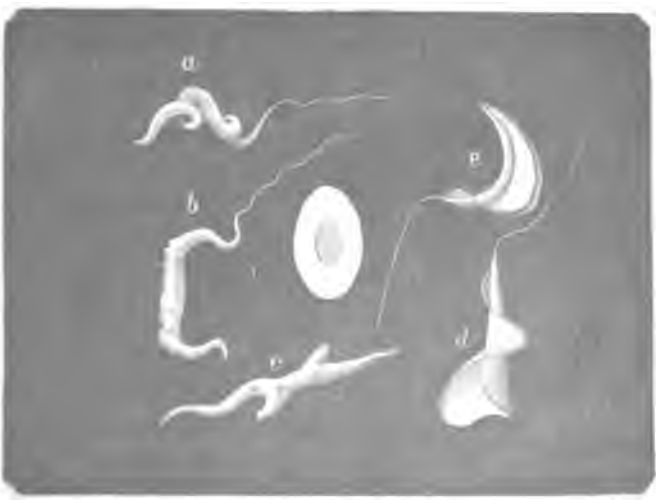


FIG. 130.—HÆMATOMONAS COBITIS.

a, First variety ; *b*, second variety ; *c*, third variety.
d, First variety in a state of diminished activity.
e, The same after treatment with osmic acid.
[After Mitrophanow.]

addition. Thus the organism consisted of a body, a spiral membrane, and a flagellum.

With higher magnification the organism appeared to consist of a refractive, strongly-contractile, protoplasmic substance, which, when death occurred, formed a shapeless mass. In the same blood two other forms were observed, one without a membrane, but having two highly-

refractive spherules in the protoplasm, and another with neither membrane nor flagellum, consisting of very granular protoplasm with several refractive spherules, and capable of protruding processes like pseudopodia.

In the blood of the German carp (*Cyprinus Carassius*) Mitrophanow describes a parasite which is perceptibly larger, and possesses an undulating membrane fastened along the edge of the long body (Fig. 131). When the body bent first towards one side and then to the other, a

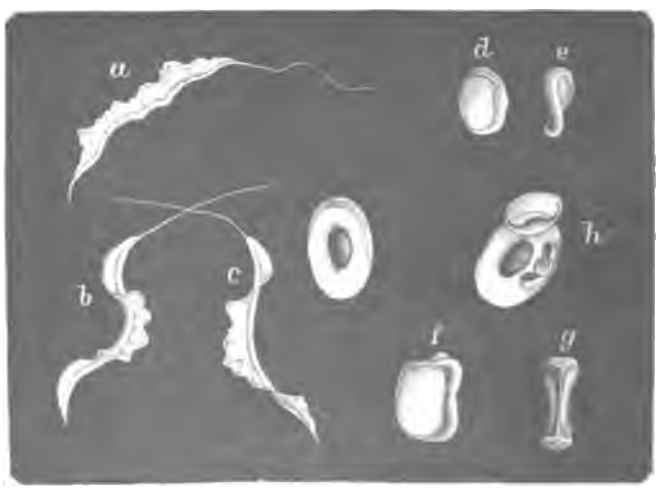


FIG. 131.—ORGANISMS IN THE BLOOD OF THE CARP.

a, *b*, *c*, *Hæmatomonas carassii*. *d*, *e*, *f*, *g*, *h*, Other organisms in the same blood [after Mitrophanow].

wave-like movement was observable at the free edge of this membrane.

In *Cobitis fossilis* these parasites were found in all the fish examined except one, and in greater numbers in the hot months. In *Cyprinus Carassius* they were only found occasionally. Mitrophanow described other varieties, which he considered were possibly not complete organisms, but developmental forms. He considered that these organisms were

infusoria between the genera *Cercomonas* and *Trichomonas*, with great similarity to the *Trichomonas* described in the Lieberkühn's glands of fowls and ducks (Eberth*).

On account of their special habitat Mitrophanow suggested a new genus (*Hæmatomonas*), defining this genus as follows:—Parasites of normal fish-blood, worm-like, actively-moving organisms, with indistinct differentiation of body parenchyma. Bodies pointed at both ends, 30 to 40 μ long and 1 to 1·5 μ wide. May possess in front a flagellum, and on one side an undulating membrane.

Species:—

Hæmatomonas cobitis.—Body provided with a spiral membrane and a flagellum at the fore-end. Parenchyma of body homogeneous. Second variety, body and flagellum only. Movement undulatory, body containing highly refractive spherules. Third variety, plasma-like body, without membrane or flagellum; quickly changes form by sending out processes laterally, and contains two to four refractive spherules. Blood of *Cobitis fossilis.*

Hæmatomonas carassii.—Long bodies, with narrow membrane attached along the whole length; less actively motile. Several forms also observed strikingly smaller than the above; many disc-shaped. Often seen attached to a red corpuscle, setting them in motion by their movements. Blood of *Cyprinus carassius.*

Quite recently the author has investigated the parasites found in the disease known as Surra, and came to the following conclusions:

In stained preparations the somewhat tapering central portion, or body, of the parasite is found to be continuous at one end with a whip-like lash, and at the other end to terminate in an acutely-pointed stiff filament, or spine-like process. Here and there, possibly from injury or want of development, the spine-like process appears to be blunted

* *Vide* Leuckart, *The Parasites of Man*, translated by Hoyle, p. 248.

or absent. By very careful focusing on the upper edge of the central portion, the author discovered the existence, much more markedly in some of the parasites than in others, of *a longitudinal membrane* with either a straight or undulating margin (Fig. 132). The membrane is attached along the body, arising from the base of the rigid filament, and becomes directly continuous at the opposite end with the flagellum. In some cases the edge only is deeply stained, giving the appearance *of a thread continuous with the flagellum*, so that one might be easily led to overlook the membrane, and imagine that the

FIG. 132.—"SURRA" PARASITES OCCURRING SINGLY AND FUSED. From preparations stained with magenta, × 1200.

flagellum arose from the opposite end of the body, at the base of the spine-like process.

Close to the base of the spine-like process a clear unstained spot is, in many parasites, easily distinguished, and at the opposite end there is, in some, the appearance of the deeply-stained protoplasmic contents having contracted within the faintly-stained cell-wall. Where the longitudinal membrane has a wavy outline the undulations are much more marked in some cases than in others. Here and there the wavy outline appears first on one side of the central portion, and then on the other, but there never is any waving outline on both sides of the same part of the body, and this is explained by careful exami-

nation, which shows that in dying the somewhat ribbon-like parasite has become doubled on itself.

Owing to the somewhat curved and twisted shape of the parasite and the curling of the flagellum, in the stained preparations, it was difficult to make exact measurements; but the average width, according to whether the membrane was visible or not, varied from 1 to 2 μ, and the length of the body from 20 to 30 μ. The flagellum was about the same length as the body.

From these observations (especially the discovery of the undulating longitudinal membrane) the author recognised a very close resemblance to Mitrophanow's descriptions, and concluded that, if we followed the classification adopted by Mitrophanow, the genus *Hæmatomonas* must

FIG. 133.—A MONAD IN RAT'S BLOOD, × 3000.

The organism is represented at partial rest, with its posterior filament impinging on a corpuscle, and showing the undulating longitudinal membrane, the long flagellum, and the refractive spherules in the granular protoplasm.

not be restricted to organisms in fish-blood. It must be expanded to include this parasite of mammalian blood, which should in that case be named *Hæmatomonas Evansi*, rather than *Spirochæta Evansi*, as proposed by Steel.

In the course of this investigation the author was led to examine the blood of rats obtainable in this country. Organisms were discovered in the blood of about 25 per cent. of common brown rats; and, after examining them with various objectives, from a $\frac{1}{5}$ dry to a $\frac{1}{25}$ oil-immersion of Powell and Lealand, the following conclusions were arrived at:—That they are polymorphic, presenting for the most part slightly tapering bodies

which terminate at one end in a stiff, immotile, acutely-pointed, flexible filament or spine-like process, and at the opposite end are provided with a long flagellum, while longitudinally attached a delicate undulating, fin-like membrane can be traced, which starts from the base of the posterior filament, and becomes directly continuous with the flagellum (Fig. 133).

With careful illumination the body is found to be distinctly granular, with one or more highly-refractive spherules. When the rapid movement is arrested the undulating membrane is distinctly visible. The best opportunity occurs for seeing this when the organism

FIG. 134.—MONADS IN RAT'S BLOOD, × 1200.

a, A monad threading its way among the blood-corpuscles; *b*, another with pendulum movement attached to a corpuscle; *c*, angular forms; *d*, encysted forms; *e* and *f*, the same seen edgeways.

comes to partial rest with its stiff filament against a corpuscle, as if to obtain a *point d'appui*, while lashing its flagellum in all directions (Fig. 134, *b*). At other times, when the parasite has impinged with its posterior extremity against a corpuscle, or the stiff filament is apparently entangled in *débris*, the movements of the organism give one the idea of its endeavouring to set itself free.

In the active state the thicker portion, or body, appears

to twist and bend from side to side with great activity. The organism can turn completely round with lightning rapidity, so that the flagellum, at one moment lashing in one direction, is suddenly observed working in the opposite direction. Then suddenly the organism makes progression, and it can be distinctly seen *to move in the direction of the flagellum, the flagellum threading its way between the corpuscles and drawing the rest of the organism after it.* By treating cover-glass preparations with osmic acid, the appearances corresponded exactly with photographs of the organisms observed by Lewis in India, so that the author has no doubt of their identity, in spite of the descriptions not completely according. A great likeness to the organisms described by Mitrophanow, and to the Surra parasite, as just described, was obvious; and after staining the rat parasites the closest examination confirmed the belief that they were morphologically identical with the stained parasites of Surra.

The cover-glasses with a thin layer of blood should be passed through the flame of a Bunsen burner in the way commonly employed for examining micro-organisms, and stained with an aqueous solution of fuchsine, methyl-violet, or Bismarck brown. The following method will, however, be found most instructive. Use freshly-prepared saturated solution of fuchsine or methyl-violet in absolute alcohol, and put a drop with a pipette on the centre of the preparation; do not disturb the drop-form for a few moments; then, before the alcohol has evaporated, wash off the excess of stain. It will be found that where the drop rested the organisms will be very deeply stained, while in the surrounding area the colour will vary in intensity.

By the effect of the different degrees of staining much may be learnt (Fig. 135). In one organism the body and entire membrane will be equally stained; in another the margin of the membrane only. In some the posterior stiff filament is stained, and at its base a darkly-stained

speck is very striking; and in other cases again the posterior filament is only faintly tinged, or an unstained spot occurs near its base.

The morphological identity of the rat and Surra parasites is thus established, and both seem morphologically identical with the organism of Mitrophanow. If we follow Mitrophanow we must, therefore, enlarge his genus of *Hæmatomonas*. The author ventures, however, to disagree with Mitrophanow in the advisability of adopting this entirely new generic name. Mitrophanow suggested this new term because of the special habitat —normal fish-blood—of the species he discovered. But

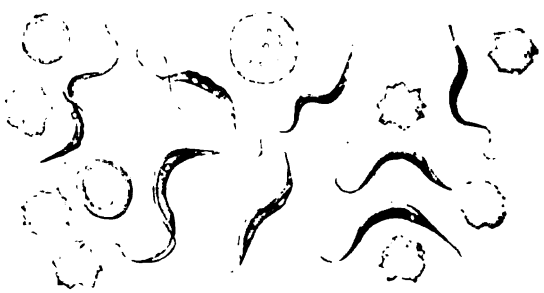


FIG. 135.—MONADS IN RAT'S BLOOD, showing membrane under different aspects, blood-corpuscles some crenated, and stained discs, × 1200.

the characteristic features of these organisms are the characteristic marks of the genus *Trichomonas*.* They are, therefore, embraced by the genus *Trichomonas*, and there is no need to create a new one. If it were not for the different description given by Mitrophanow of the organism in the blood of *Cobitis fossilis*, the author would be inclined to say that all these organisms belonged to one and the same species, which might well be named *Trichomonas sanguinis*. The monads in the rat and the Surra parasite have been shown to be morphologically identical with each other, and both, as far as one can

* *Vide* Leuckart, *The Parasites of Man*, translated by Hoyle, 1886.

judge from the description, are morphologically identical with the monad in the blood of the carp. We have, however, seen that the organism in Surra is believed to be pathogenic, and too much stress must not be laid on morphological identity. There is strong evidence in favour of believing in its pathogenic properties; but, at the same time, it must be borne in mind that the organism has never been *isolated apart from the blood*, and the disease then produced by its introduction into healthy animals. It is quite possible that the parasites in Surra are only associated with the disease, the impoverished blood affording a suitable nidus for their development, while the contaminated water may be the common source of the organism and of the disease. On the other hand, the organism in the rat is found in apparently perfectly healthy, well-nourished animals.

APPENDIX C.

EXAMINATION OF AIR.

THE air, as is well known, contains in suspension mineral, animal, and vegetable substances. The mineral world is represented by such substances as silica, silicate of aluminium, carbonate and phosphate of calcium, which may be raised from the soil by the wind, and particles of carbon, etc., which gain access from accidental sources. Belonging to the animal kingdom we find the *débris* of perished creatures as well as sometimes living animals. The vegetable world supplies *micrococci*, *bacilli*, and other forms of the great family of *bacteria*, spores of other fungi, pollen seeds, parts of flowers, and so forth. The air of hospitals and sick rooms has been found to be especially rich in vegetable forms, *e.g.*, fungi and spores have been observed as present in particularly large numbers in cholera wards, spores of Tricophyton have been discovered in the air of hospitals for diseases of the skin, and achorion in wards with cases of favus. The tubercle-bacil-

lus is said to have been detected in the breath of patients suffering from phthisis.

These points indicate that, in addition to the interest for the microbiologist, considerable importance from a hygienic point of view must be attached to the systematic examination of the air. Especially a knowledge of the microbes which are found in the air of marshy and other unhealthy districts, and in the air of towns, dwellings, hospitals, workshops, factories, and mines, will be of practical value.

Miquel,* who has particularly studied the bacteria in the air, has found that their number varies considerably. The average number per cubic metre of air for the autumn quarter at Montsouris is given as 142, winter quarter 49, spring quarter 85, and summer quarter 105. In air collected 2,000 to 4,000 metres above the sea-level, not a single bacterium or fungus spore was furnished, while in 10 cubic metres of air from the Rue de Rivoli (Paris) the number was computed at 55,000.

The simplest method for examining the organisms in air consists in exposing plates of glass or microscopic slides coated with glycerine, or a mixture of glycerine and glucose which is stable, colourless, and transparent. Nutrient gelatine spread out on glass plates (p. 81) may be exposed to the air for a certain time, and then put aside in damp chambers for the colonies to develop. Sterilised potatoes prepared in the usual way (p. 82) may be similarly exposed. In both the last mentioned methods separate colonies develop, which may be isolated as already described, and pure cultivations carried on in various other nutrient media (p. 79). Nutrient gelatine has also been employed in the special methods of Koch and Hesse.

Koch's Apparatus.—This consists of a glass jar about six inches high, the neck of which is plugged with cotton wool. In the interior is a shallow glass capsule, which can be removed by means of a brass lifter. The

* Miquel, *Organismes vivants de l'atmosphère.*

whole is sterilised by exposure to 150° C. for an hour in the hot-air steriliser. The nutrient gelatine in a stock-tube is liquefied, and the contents emptied into the glass capsule. The jar is exposed to the air to be examined for a definite time, the cotton wool plug replaced, and the apparatus set aside for the colonies to develop.

Hesse's Apparatus (Fig. 136).—The advantage of

FIG. 136.—HESSE'S APPARATUS.

this apparatus consists in that a known volume of air can be examined. A glass cylinder, 70 cm. long and 3·5 cm. in diameter, is closed at one end by an india-rubber cap, perforated in the centre. Over this fits another cap, which is not perforated. The opposite end of the cylinder is closed with a caoutchouc stopper, perforated to admit a glass tube plugged with cotton wool. The tube can be connected by means of india-rubber tubing with an

aspirating apparatus. This apparatus consists of a couple of litre-flasks, suspended by hooks from the tripod stand which supports the whole apparatus. The cylinder, caps, and plug, are washed with solution of corrosive sublimate, and then with alcohol. After being thus cleansed, 50 ccm. of nutrient gelatine are introduced, and the whole sterilised by steaming for half an hour for three successive days. After the final sterilisation the cylinder is rotated on its long axis, so that the nutrient medium solidifies in the form of a coating over the whole of the interior. When required for use, the cotton-wool plug is removed from the small glass tube, and the latter connected with the upper flask by means of the india-rubber tubing.

The apparatus is placed in the air which is to be examined, the outer india-rubber cap removed from the glass cylinder, and the upper flask tilted until the water begins to flow into the lower one. The emptying continues by syphon action, and air is drawn in along the cylinder to replace the water. When the upper flask is empty, the position of the two is reversed, and the flow again started. When a sufficient volume has been drawn through the cylinder the outer cap and the cotton-wool plug are replaced, and it is set aside for the colonies to develop. As an example, twenty-five litres of air from an open square in Berlin gave rise to three colonies of bacteria and sixteen moulds; on the other hand, two litres from a school-room just vacated by the scholars gave thirty-seven colonies of bacteria and thirty-three moulds.

Various forms of "aeroscopes" and "aeroniscopes" have from time to time been employed. Pouchet's aeroscope consists of a small funnel, drawn out to a point below which is a glass slip coated with glycerine. The end of the funnel and the glass slip are enclosed in an air-tight chamber, from which a small glass tube passes out connected by india-rubber tubing with an aspirator. The air passing down the funnel strikes upon the glycerine,

which arrests any solid particles. For a description of the more exact apparatus employed by Maddox, Cunningham, and Miquel reference should be made to the writings of these authors, and particularly to the treatise published by the last-named.

APPENDIX D.

EXAMINATION OF SOIL.

SURFACE-SOIL, or mould, is exceedingly rich in bacteria. Miquel, *e.g.*, has computed that there exists in a gramme of soil an average of 750,000 germs at Montsouris, 1,300,000 in the Rue de Rennes, and 2,100,000 in the Rue de Monge. As agents of putrefaction and fermentation they play a very important *rôle* in the economy of nature, but there exist in addition bacteria in the soil which are pathogenic in character. Pasteur has succeeded in isolating from the earth the bacillus of anthrax, and sheep, sojourning upon a plot of ground where animals which have died of anthrax had been buried, succumbed to the disease. Pasteur considered that the spores were conveyed by worms from buried beasts to the surface soil. The bacillus of malignant œdema is also present in soil, and Nicolaier has cultivated a bacillus from earth which produced tetanus in mice, rabbits, guinea-pigs, and other animals.

To obtain a cultivation of the microbes in soil a sample of the latter must be first dried and then triturated. It may then be shaken up with distilled water, and from this a drop transferred to sterilised bouillon. The employment of solid media is, however, much more satisfactory: A sample of earth is collected, dried, and triturated, and a small quantity sprinkled over the surface of nutrient gelatine prepared for a plate-cultivation. In another method the gelatine is liquefied in a

test-tube, the powder added, and, in the usual way, distributed throughout the medium, which is then poured out upon a glass plate. Just in the same way the dust which settles from the air in houses and hospitals, or food substances in powder, may be distributed over nutrient gelatine, and the micro-organisms which develop studied, both as to their morphological and biological characteristics.

APPENDIX E.

EXAMINATION OF WATER.

As in the case of air, so, too, in that of water a knowledge of the micro-organisms which may be present is not only of interest to the microbiologist, but of the greatest importance in practical hygiene. Common putrefactive bacteria and vibrios may not be hurtful in themselves, but they indicate the probability of the presence of organic matter in some of which there may be danger.*

The Microzyme Test, which was introduced for their detection, consisted in adding three or four drops of the sample of water to 1 or 2 ccm. of Pasteur's fluid, the nourishing fluid having been previously boiled in a sterilised test-tube. If the microzymes or their germs existed in the water, the liquid in a few days became milky from the presence of countless bacteria. This test is of no real value, for it does little more than indicate that bacteria were present, which we may accept as being present in ice and all ordinary water. On the other hand, the bacteriological test of Professor Koch is a most valuable addition to the usual methods of water-analysis. It enables us not only to detect the presence of bacteria, but to ascertain approximately their number, and to study very minutely their morphological and biological charac-

* Parkes, *Manual of Practical Hygiene.* 1883.

teristics. The importance of a thorough acquaintance with the life-history of the individual micro-organisms cannot be too strongly insisted upon. For example, by such means the spirillum of Asiatic cholera can be distinguished from other comma-shaped organisms, and inasmuch as its presence may be an indication of contamination with choleraic discharges, such water should be condemned for drinking purposes, even though we may not yet be in a position to affirm that the microbe is the cause of the disease. The test, in short, consists in making plate-cultivations of a known volume of water, counting the colonies which develop, isolating the micro-organisms, and studying the characters of each individual form.

Collection and Transport of Water Samples.—Erlenmeyer's conical flasks of about 100 ccm. capacity may be employed with advantage for collecting the samples of water. They are cleansed, plugged, and sterilised in the hot-air steriliser. When required for use, the plug is removed and held between the fingers, which must not touch the part which enters the neck of the flask. About 30 ccm. of the water to be examined are introduced into the flask, and the plug must be quickly replaced and covered with a caoutchouc cap. If collected from a tap, the water should first be allowed to run for a few minutes, and the sample should be received into the flask without the neck coming into contact with the tap. From a reservoir or stream the flasks may be filled by employing a sterilised pipette. During transport contact between the water and cotton-wool plug must be avoided, and if likely to occur the sample must be collected and forwarded in a Sternberg's bulb (p. 31).

Examination by Plate Cultivation.—The apparatus for plate-cultivation should be arranged as already described. Crushed ice may be added to the water in the glass dish to expedite the setting of the gelatine, so that the plate may be transferred as quickly as possible to the damp-chamber. The caoutchouc cap

is removed from the flask, and the cotton-wool plug singed in the flame to prevent contamination from adventitious germs on the outside of the plug. The flask is then held slantingly in the hand, and the plug twisted out and retained between the fingers. With a graduated pipette a drop of the sample is transferred to a tube of liquefied nutrient gelatine, and the plug of the flask and tube quickly replaced. If the water is very impure, it may be necessary to first dilute the sample with sterilised water. The inoculated tube must be gently inclined backwards and forwards and rolled as already explained, to distribute the germs throughout the gelatine (p. 75), and the gelatine finally poured on a plate.

FIG. 137.—APPARATUS FOR ESTIMATING THE NUMBER OF COLONIES IN A PLATE-CULTIVATION.

When the gelatine has set, the plate is transferred to a damp chamber, which should be carefully labelled and set aside in a place of moderate temperature. In about two or three days the cultivation may be examined. In some cases the colonies may be counted at once; more frequently they are so numerous that the plate must be placed on a dark background, and a special process resorted to. A glass-plate, ruled by horizontal and vertical lines into centimetre squares, some of which are again subdivided into ninths, is so arranged on a wooden frame that it can cover the nutrient gelatine-plate without touching it (Fig. 137). A lens is added to assist in discovering minute colonies. If then the colonies are very numerous,

the number in some small divisions is counted, if less in some large ones, and an average is obtained from which the number of colonies on the entire surface is calculated. A separate calculation of the *liquefying* colonies should be also made, and their number, as well as the total number of colonies present in 1 ccm. of the sample, recorded. Any peculiar macroscopical appearances, colour, etc., should be noted, and then the microscopical appearances of the colonies studied. Lastly, examination of the individual organisms should be made by cover-glass-preparations, and by inoculation of nutrient gelatine, potatoes, and other media.

Examination by Test-Tube Cultivation.—A drop of the sample of water may also be added to liquefied nutrient gelatine in a tube, the organisms distributed as already explained (p. 75,) and the gelatine allowed to solidify in the tube. A rough comparison of water samples may be made in this way.

Microscopic Examination.—A drop of the water may be mounted and examined in the way described under drop-cultivations (p. 94), or a drop is allowed to evaporate on a cover-glass placed under a bell-glass. This is then passed three times through the flame, and stained in the usual manner. The examination of rain water, drinking water, tap water, sea water, various liquids and infusions, etc., by these methods opens up a wide field for research. Pettenkofer has shown that impregnation with carbonic acid of water containing many bacteria diminishes the number of the latter. The examination of waters before and after filtration, or after addition of chemical substances, are matters which require further investigation.

APPENDIX F.

CHRONOLOGICAL BIBLIOGRAPHY.

A. METHODS.

B. MORPHOLOGY AND CLASSIFICATION.

C. GENERAL BIOLOGY.

D. ZYMOGENIC SAPROPHYTES AND FERMENTATION.

E. CHROMOGENIC SAPROPHYTES

F. SIMPLE SAPROPHYTES.

G. PTOMAINES AND PUTREFACTION.

H. ANTISEPTICS AND DISINFECTANTS.

I. IMMUNITY.

J. BACTERIA ASSOCIATED WITH DISEASES IN MAN AND ANIMALS —

I. ACTINOMYCOSIS.
II. ACUTE YELLOW ATROPHY.
III. ANTHRAX.
IV. CATTLE PLAGUE.
V. CEREBRO-SPINAL MENINGITIS.
VI. CHICKEN-CHOLERA.
VII. CHOLERA.
VIII. DENTAL CARIES.
IX. DIPHTHERIA.
X. ERYSIPELAS.
XI. ENDOCARDITIS.
XII. GLANDERS.
XIII. GONORRHŒA.
XIV. HYDROPHOBIA.
XV. LEPROSY.
XVI. MALARIA.
XVII. MALIGNANT ŒDEMA.
XVIII. MEASLES.
XIX. OPHTHALMIC DISEASES.
XX. OSTEO-MYELITIS.
XXI. PLEURO-PNEUMONIA.
XXII. PNEUMONIA.
XXIII. PUERPERAL FEVER.
XXIV. PYÆMIA AND SEPTICÆMIA.
XXV. RELAPSING FEVER.
XXVI. RHINOSCLEROMA.
XXVII. SCARLATINA.
XXVIII. SWINE-ERYSIPELAS.
XXIX. SWINE-TYPHOID.
XXX. SYMPTOMATIC ANTHRAX.
XXXI. SYPHILIS.
XXXII. TETANUS.
XXXIII. TUBERCULOSIS.
XXXIV. TYPHOID FEVER.
XXXV. VARIOLA AND VACCINIA.
XXXVI. YELLOW FEVER.

K. BACTERIA IN THE AIR, IN SOIL, AND IN WATER.

(A) *METHODS.*

1852 PERTY.—Zur Kenntniss Kleinster Lebensform.

1867 PASTEUR.—Etudes sur la Bière.

1873 KLEBS.—Ueber Fractionirte Cultur. Archiv f. Exp. Pathol., Bd. I.

1875 BREFELD.—Verhandl. d. Physik. Med. Ges. in Würzburg.

COHN.—Beiträge zur Biologie der Pflanzen, Bd. I., Heft 3.

1876 COHN.—Beiträge zur Biologie der Pflanzen, Bd. II., Heft 2.

1877 SCHÄFER.—Course of Practical Histology.

KOCH.—Verfahren z. Untersuch., z. Conservir. und Photographir. der Bakterien. Beiträge z. Biol. d. Pflanzen, Bd. II., Heft 3.

1878 KOCH.—Untersuchungen über Wundinfections Krankheiten.

1879 SALOMONSEN.—Zur Isolation Differenter Bakterien. Bot. Zeitung, No. 39.

BLANCHARD.—Rev. Inter. Sci., III.

1880 SALOMONSEN.—Eine Einfache Meth. z. Reincultur Versch. Fäulniss Bakterien. Bot. Zeit., No. 28.

EHRLICH.—Zeitschr. f. Klin. Med., Bd. I.

1881 EHRLICH.—Zeitschr. f. Klin. Med., Bd. II., Heft 3.

CARPENTER.—The Microscope and its Revelations. 6th Edition.

KOCH.—Zur Untersuchung von Pathogenen Organismen. Mitth. aus d. K. Gesundheits Amt, Bd. I.

BREFELD.—Bot. Untersuch. über Schimmelpilze, Bd. IV.

DUCLAUX.—Ferments et Maladies.

1882 KOCH.—Biol. Klin. Woch., No. 15.

EHRLICH.—Deut. Med. Woch., No. 19.

1882 FEHLEISEN.—Ueber Neue Method. der Untersuch. u. Cultur Pathogen. Bakterien. Physik. Med. Ges. zu Würzburg.

BÜCHNER.—In Nägeli's Untersuch. über Niedere Pilze. Munich.

1883 ALMQUIST.—Hygeia, XLV. Stockholm.

BEHRENS.—Hilfsbuch zur Ausführung Mikroskop. Untersuch.

HAZLEWOOD.—American Monthly Microscop. Journ.

BREFELD.—Die Künstl. Kultur Parasitischer Pilze. Botan. Unters. über Hefenpilze, Bd. V.

FLÜGGE.—Handbuch der Hygiene und der Gewerbe Krankheiten.

1884 MAGNIN and STERNBERG.—Bacteria.

FRIEDLÄNDER.—Microscopische Technik. 1st Edition.

BÜCHNER.—Ueber das Verhalten der Spaltpilzsporen zu den Anilinfarben. Aerztl. Intelligenzbl., No. 33.

ORTH.—Patholog. Anatom. Diagnostik.

PLAUT.—Färbungs Methode z. Nachweis. der Micro-organismen. 1st Ed.

BAUMGARTEN.—Beitr. zur Darstellungsmethode d. Tuberkel Bacillen. Zeitschr. f. Wissensch. Mikr.

KOCH.—Mitth. a. d. Kais. Ges. Amt, Bd. II.

GRAM.—Ueber die Isolirte Färbung der Schizomyceten. Fortsch. d. Med., II., No. 6.

1885 HUEPPE.—Die Methoden der Bakterien Forschung.

CORNIL ET BABES.—Les Bactéries.

GIBBES.—Practical Histology and Pathology.

HAUSER.—Ueber Fäulnissbacterien. Mit 15 Tafeln in Lichtdruck.

KLEIN.—Micro-organisms and Disease.

LEE.—The Microtomist's Vade Mecum.

1885 MALLEY.—Photomicrography.
PLAUT.—Färbungs Methoden. 2nd Edition.
WOODHEAD and HARE.—Pathological Mycology.
BIZZOZERO ET FIRKET.—Manuel de Microscopie Clinique.
JOHNE.—Ueber die Kochschen Reinculturen.
BORDONI UFFREDUZZI.—Microparasitici.
BANTI.—Manuale di Tecnica Batteriologica.
DOLLEY.—Technology of Bacteria Investigation.
1886 HUEPPE.—Die Methoden der Bakterien Forschung. 3rd Ed.
HUEPPE.—The Methods of Bacteriological Investigation. Translated by Biggs.
HÜBER AND BREKER.—Die Path. Histolog. und Bacteriologischen Untersuch. Methoden.
KLEIN.—Micro-organisms and Disease. 3rd Ed.
CORNIL AND BABÈS.—Les Bactéries. 2nd Edition.
CROOKSHANK.—Manuel Pratique de Bactériologie, traduit par Bergeaud. Avec 4 Photomicrographies.
PLAUT.—Fortschr. d. Med.
EISENBERG.—Bakteriologische Diagnostik.
ESMARCH.—Zeitschrift f. Hygiene.
HUEPPE.—Bakteriologische Apparate. Deut. Med. Woch.

(B) *MORPHOLOGY AND CLASSIFICATION.*

1838 EHRENBERG.—Die Infusionsthierchen als Volkom. Organism.
1841 DUJARDIN.—Histoire Naturelle des Zoophytes.
1866 DE BARY.—Morph. und Phys. der Pilze.
HALLIER.—Die Pflanzlichen Parasiten.
1872 COHN.—Beiträge zur Biologie der Pflanzen, Bd. I.
1873 LISTER.—Quart. Journ. Microscop. Science.
LANKESTER.—Quart. Journ. Microscop. Science.

1874 BILLROTH.—Unters. über d. Veg. Form der Coccobacteria septica.
1875 COHN.—Beiträge z. Biol. d. Pflanzen.
DALLINGER AND DRYSDALE. Monthly Miscroscop. Journ.
1877 LEUNIS.—Synopsis d. Pflanzenkunde. Hanover.
NÄGELI.—Die Niederen Pilze.
1879 VAN TIEGHEM.—Compt. Rend.
1881 BREFELD.—Botanische Untersuch. über Schimmelpilze, Heft 1.
KOCH.—Mitheil aus d. K. Ges. Amt, Bd. I.
RABENHORST.—Kryptogamenflora. Bd. I., Pilze, von Winter.
1882 SACHS.—Text-Book of Botany.
1883 FLÜGGE.—Handbuch der Hygiene.
NEELSEN.—Neuere Ansichten über d. Systematik der Spaltpilze. Biol. Centralbl., III., No. 18.
VAN TIEGHEM.—Traité de Botanique.
1884 DE BARY.—Verg. Morph. und Biolog. der Pilze, Mycetozoen, und Bacterien.
GROVE.—Synopsis of the Bacteria and Yeast Fungi.
MARPMANN.—Die Spaltpilze.
MILLER.—Deut. Med. Woch.
1885 CORNIL AND BABÈS.—Les Bactéries.
FISCH.—Die Systemat. Stellung der Bacterien. Biolog. Centralbl., V.
ZOPF.—Die Spaltpilze.
A. DE BARY.—Vorlesungen über Bacterien.
BIEDERT.—Virch. Archiv, Bd. 100.
HAUSER.—Ueber Fäulniss Bacterien.
1886 HUEPPE.—Die Formen der Bakterien.
BAUMGARTEN.—Lehrbuch der Pathologischen Mykologie.
FLÜGGE.—Die Micro-organismen. 2nd Ed.
LUTZ.—Fortschr. d. Med.

(C) *GENERAL BIOLOGY.*

1877 NÄGELI.—Die Niederen Pilze. München.
1879 COHN U. MENDELSOHN.—Ueber Einwirkung des Elektrischen Stromes auf die Vermehrung d. Bakterien. Beitr. z. Biol. d. Pflanzen, Bd. III., Heft 1.
NENCKI.—Virchow's Archiv.
1880 NENCKI.—Beiträge zur Biol. der Spaltpilze. Leipzig.
1881 TUMAS.—St. Petersb. Med. Wochenschr.
ENGELMANN.—Arch. f. d. Ges. Physiologie, Bd. 26.
1882 ENGELMANN.—Botan. Zeitg.
NÄGELI.—Unters. über Niedere Pilze. München.
1884 REGNARD.—Rech. sur l'Influence de très-hautes Pressions sur les Org. Vivants. Compt. Rend., T. 98, p. 744.
CORTES.—De l'Action des Hautes Pressions sur les Phénomènes de la Putréfraction, etc. Compt. Rend. T. 99, p. 385.
NENCKI.—Ueber das Eiweiss der Milzbrandbacillen. Ber. d. Deutschen Chem. Gesellsch. S. 2605.
1885 DUCLAUX.—Influence de la Lumière sur la Vitalité des Germes de Microbes. Compt. Rend.
MITTENZWEIG.—Die Bakt. Aetiolog. der Infect. K.
1886 v. FODOR.—Bakterien im Blute lebender Thiere. Archiv. f. Hygiene.
HAUSER.—Archiv. f. Exper. Patholog. u. Pharmacologie.
WYSSOKOWITSCH.—Zeitschrift. f. Hygiene.
PAVY.—Relation of Bacteria to Disease. Harveian Oration. Brit. Med. Journ.
BORDONI-UFFREDUZZI.—Fortschr. d. Med.
ARLOING.—Archiv. de Physiol.
WATSON-CHEYNE.—Brit. Med. Journ.
LIBORIUS.—Zeitschrift. f. Hygiene.
DOWNES.—Action of Sunlight on Micro-org. Proc. Roy. Soc.

(D) *ZYMOGENIC SAPROPHYTES AND FERMENTATION.*

1860 MÜLLER.—Journ. f. Prakt. Chem.
PASTEUR.—Annal. de Chim. et de Phys., III. Sér., T. 58.
PASTEUR.—Compt. Rend., T. 50.

1861 LIEBIG.—Verhandl. der Münchener Akad. d. Wiss.
PASTEUR.—Bull. de la Soc. Chim.
PASTEUR.—Compt. Rend., T. 52.

1862 PASTEUR.—Ann. de Chim. et de Phys., T. 64.
MONOYER.—Thèse de Strassburg.

1863 PASTEUR.—Compt. Rend., T. 56.

1864 PASTEUR.—Compt. Rend.
VAN TIEGHEM.—Compt. Rend., T. 58, p. 210.
TYNDALL.—Compt. Rend., T. 58.

1865 DUCLAUX.—Thèses présentées à la Faculté de Paris.
BÉCHAMP.—Compt. Rend., T. 60, p. 445.
TRÉCUL.—Ueber Bacillus Amylobacter. Compt. Rend., T. 61.

1866 PASTEUR.—Etudes sur le Vin.

1867 HALLIER.—Gährungserscheinungen.
TRÉCUL.—Compt. Rend., T. 65.
TRÉCUL.—Ann. des Sc., Sér. 7, T. 7.

1869 KARSTEN.—Chemismus der Pflanzenzelle.
LIEBIG.—Verhandl. der Münchener Akad. d. Wiss., 5 Nov.

1870 LIEBIG.—Ueber Gährung, Quelle der Muskelkraft und Ernährung. Leipzig u. Heidelberg.

1871 PASTEUR.—Compt. Rend.
DUBRUNFAUT.—Compt. Rend., T. 73.
HOPPE-SEYLER.—Medic.-Chem. Untersuchungen, Heft 4.

1872 PASTEUR.—Compt. Rend.
DUMAS.—Compt. Rend., T. 75, Nr. 6.
LEX.—Centralbl. f. d. Med. Wiss., S. 291.

1873 FITZ.—Berichte d. Chem. Ges., Bd. 6, S. 48.
HOFMANN.—Aerztl. Verein zu Wien, Mai.
HOFMANN.—Allgem. Med. Centralbl., S. 605.
1874 SCHÜTZENBERGER.—Die Gährungserscheinungen.
DUMAS.—Ann. de Chim. et de Phys.
BREFELD.—Landwirthsch. Jahresber., Bd. 3.
SCHEIBLER.—Ueber die Natur des Froschlaich. Zeitschr. f. Rübenzuckerindustrie.
FELTZ ET RITTER.—Journ. de l'Anat. et Phys.
HILLER.—Centralbl. f. d. Med. Wiss., S. 53.
VAN TIEGHEM.—Compt. Rend., T. 58.
MUSCULUS.—Ber. d. Chem. Ges., S. 124.
MUSCULUS.—Compt. Rend., T. 78.
1875 POPOFF.—Botan. Jahresber.
BREFELD.—Landwirthsch. Jahresber., Bd. 4.
COLIN.—Bull. de l'Acad. de Méd.
1876 PASTEUR.—Bull. de l'Acad. de Méd., No. 27.
PASTEUR ET JOUBERT.—Compt. Rend., T. 83.
PASTEUR.—Etudes sur la Bière.
A. MAYER.—Lehrbuch der Gährungschemie. 2 Aufl.
FLECK.—Ber d. Chem. Centralst. Dresden.
BREFELD.—Landwirthsch. Jahresber., Bd. 5.
1877 LISTER.—The Cause of Putrefaction and Lactic Fermentation. The Pharmac. Journ. and Transact.
HARZ.—Grundzüge der alkoholischen Gährungslehre.
1878 CIENKOWSKI.—Die Gallertbildungen d. Zuckerrübensaftes.
BOUTROUX.—Sur la Fermentation Lactique. Compt. Rend., T. 86.
FITZ.—Berichte d. Chem. Ges., Bd. 10, p. 216.
1879 FITZ.—Berichte d. Chem. Ges., Bd. 11, pp. 42 and 498; and Bd. 12, p. 474.
RICHET.—Compt. Rend., T. 88.
NÄGELI.—Theorie der Gährung. München.

1879 VAN TIEGHEM.—Développement du Spirillum Amyliférum. Bull. de la Soc. Bot. de France, and Sur la Fermentation de la Cellulose, pp. 25—30.

VAN TIEGHEM.—Compt. Rend., T. 88.

VAN TIEGHEM.—Identité du Bacillus Amylobacter et du Vibrion Butyrique de Pasteur. Compt. Rend., T. 89.

1880 FITZ.—Berichte d. Chem. Ges., Vol. 13, p. 1309.

PRAZMOWSKI.—Untersuchungen über die Entwicklungsgeschichte und Fermentwirkung einiger Bakterien.

VAN TIEGHEM.—Compt. Rend.

1881 BÉCHAMP.—Compt. Rend., T. 93.

TYNDALL.—Essays on the Floating Matter of the Air.

ERIKSSON.—Unters. aus d. Botan. Institut in Tübingen, Heft 1.

KERN.—Ueber ein neues Milchferment aus dem Kaukasus. Bull. de la Soc. Imp. des Naturalistes de Moskau, No. 3.

V. JACKSCH.—Studien über den Harnstoffpilz. Zeitschr. f. Physiol. Chemie, Bd. 5.

1882 FITZ.—Berichte d. Chem. Ges., Bd. 15, p. 857.

1883 GUIARD.—Thèse de Paris.

TAPPEINER.—Celluloseverdauung. Fortschr. d. Med., I., 151; II., 377, 416.

FITZ.—Berichte d. Chem. Ges., Bd. 16, p. 844.

1884 HUEPPE.—Unters. üb. die Zersetzungen der Milch durch Mikroorganismen. Mitth. a. d. Ges. Amt., Bd. II.

HUEPPE.—Deut. Med. Woch.

KRANNHALS.—Ueber das Kumysähnliche Getränk Kephir. Deut. Arch. f. Klin. Med., Bd. 35.

FITZ.—Berichte d. Chem. Ges., Bd. 17, p. 1188.

LADUREAU.—Sur le Ferment Ammoniacal. Compt. Rend., T. 99, p. 877.

1884 VAN TIEGHEM.—Développement de l'Amylobacter dans les Plantes à l'Etat de Vie Normale. Compt. Rend.

1885 LÉPINE ET ROUX.—Compt. Rend., T. 101.

LEUBE.—Ueber die Ammoniakalische Harngährung. Virch. Arch., Bd. 100, S. 540.

BILLET.—Sur le Bacterium Ureæ. Ib., Bd. 100, p. 1252.

SHERIDAN LEA.—Journ. of Physiology.

(E) *CHROMOGENIC SAPROPHYTES.*

1833 HERMSTÄDT.—Ueber die Blaue und Rothe Milch.

1838 STEINHOFF.—Ueber das Blauwerden der Milch. Neue Ann. d. Mecklenb. Landw. Ges.

1839 EHRENBERG.—Micr. Prodigiosus. Verhandl. d. Berl. Acad.

1850 CHABERT ET FROMAGE.—D'une Altération du Lait de Vache, désignée sous le Nom du Lait Bleu.

1852 GIELEN.—Mag. f. Ges. d. Thierheilkunde.

1860 FORDOS.—Compt. Rend. de l'Acad. de Sc.

1862 LÜCKE.—Arch. f. Klin. Chir.

1863 EBERTH.—Centralbl. f. d. Med. Wissensch.

1868 MOSLER.—Ueber Blaue Milch und durch deren Genuss herbeigeführte Krankheiten. Virchow's Archiv, Bd. 43.

1872 SCHRÖTER.—Ueber einige von Bakterien gebildete Pigmente. Cohn, Beitr. z. Biol. der Pflanzen, Bd. I., Heft 2.

1873 LANKESTER.—On a Peach-coloured Bacterium. Quart. Journ. of Micr. Sc., Vol. 13.

1875 FRANK.—Cohn's Beitr. z. Biol. d. Pflanzen, Bd. I., Heft 3.

GIRARD.—Unters. über Blauen Eiter. Chirurg. Centralbl., II.

1875 KLEIN.—Quart. Journ. of Micr. Sc., Vol. 15.
1876 LANKESTER.—Further Observations on a Peach- or Red-coloured Bacterium. Quart. Journ. of Micr. Sc.
1877 GIARD.—Revue des Sc., T. 5.
1879 WERNICH.—Cohn's Beiträge zur Biol. d. Pflanzen, Bd. III., Heft 1.
COHN U. MIFLET.—Cohn's Beiträge zur Biol. d. Pflanzen, Bd. III., Heft 1.
1880 VAN TIEGHEM.—Bull. de la Soc. Bot. de France.
NEELSEN.—Cohn's Beitr. z. Biologie d. Pflanzen, Bd. III., Heft 2.
1882 BABÈS.—Vom Rothen Schweiss. Biolog. Centralbl., Bd. 2.
GESSARD.—De la Pyocyanine et de son Microbe.
1884 CHARRIN.—Communication faite à la Société Anatomique.
HUGUES.—Le Lait Bleu. Echo Vétérinaire.

(F) *SIMPLE SAPROPHYTES.*

1833 KÜTZING.—Sphaerotilus Natans. Linnæa, 8.
1840 OERSTED.—Naturhist. Tidsskrift, Bd. III.
1842 GOODSIR.—Edinb. Med. and Surg. Journ.
1845 WEISSE.—Monas Okenii. Bull. Phys.-Mathemat. de St. Pétersbourg, III.
1847 HELLER.—Heller's Arch. f. Chemie.
1858 EBERTH.—Virchow's Archiv, Bd. 13.
ITZIGSOHN.—Virchow's Archiv, Bd. 13.
1859 WELCKER.—Zeitschr. f. Ration. Med.
1861 MUNK.—Virch. Arch., Bd. 22.
1862 PASTEUR.—Ann. de Chim. et de Phys., T. 64.
1864 FRIEDREICH.—Beiträge zur Kenntniss der Sputa. Virch. Arch., Bd. 30.
MUNK.—Med. Centralbl.

1865 COHN.—Ueber zwei Neue Beggiatoen. Hedwigia.
1866 SURINGAR.—Arch. Neerland.
SURINGAR.—Ueber den Zellenbau der Sarcina. Bot. Zeit.
1867 LÜDERS.—Ueber Abstammung u. Entwicklung des Bacterium Termo.
1871 LOSDORFER.—Med. Jahrb., Heft 3.
1872 COHN.—Beitr. z. Biol. d. Pflanzen, Bd. I.
1873 LANKESTER.—Quart. Journ. of Microscop. Sc.
1874 CASPARY.—Schriften der Physik. Oekon. Ges. zu Könisberg, Bd. 15.
EWART.—Proceedings of the Roy. Soc.
1875 EIDAM. Cohn's Beitr. zur. Biol. d. Pflanzen.
DALLINGER AND DRYSDALE.—Monthly Microsc. Journ.
1876 LANKESTER.—Quarterly Journ. of Microscop. Sc.
1877 HEIMER. — Ueber Pneumonomycosis Sarcinica. Deut. Arch. f. Klin. Med.
1878 DALLINGER.—Journ. of the Roy. Micro. Soc. London.
GEDDES AND EWART.—On the Life-History of Spirillum. Proceed. of the Roy. Soc. of London.
BREFELD.—Bacillus Subtilis. Ges. Nat. Freunde.
1879 ZOPF.—Ueber Crenothrix Polyspora. Berlin.
1882 ZOPF.—Bacterium Merismopedioïdes. Sitz.-Ber. d. Botan. Ver. d. Prov. Brandenb. Juni.
ENGELMANN.—Bacterium Photometricum. Unters. aus. d. Phys. Laborat. zu Utrecht. Pflüger's Arch. f. d. Ges. Physiol., Bd. 30, S. 95.
GIARD.—Sur le Crenothrix Kühniana. Compt. Rend.
1883 KURTH.—Bacterium Zopfii. Ber. d. Deutsch. Bot. Ges. Botanische Zeitg.
1884 VANDEVELDE.—Studien zur Chemie des Bacillus Subtilis. Zeitschr. f. Phys. Chemie, Bd. 8.
MÜLHÄUSER. — Ueber Spirillen. Virch. Arch., Bd. 97.

1885 FALKENHEIM.—Arch. f. Exp. Patholog. u. Pharmakol., Bd. 19.

BILLET.—Sur la Formation des Spores chez le Cladothrix Dichotoma. Compt. Rend., T. 100.

1886 BORDONI-UFFREDUZZI.—Ueber d. biol. Eigensch. d. norm. Hautmikrophyt. Fortsch. d. Med.

(G) *PTOMAINES AND PUTREFACTION.*

1866 HEMMER.—Exper. Studien über d. Wirkung Faulender Stoffe.

SCHWENINGER.—Ueber d. Wirkung Faulender Org. Substanzen.

v. RAISON.—Zur Kenntniss der Putriden Intoxication.

BERGMANN.—Das Putride Gift.

1868 BERGMANN U. SCHMIEDEBERG.—Med. Centralbl.

1869 ZÜLZER U. SONNENSCHEIN.—Berl. Klin. Woch.

1872 BERGMANN.—Deut. Zeitschr. f. Chirurgie, Bd. I.

RAVITSCH.—Zur Lehre von der Putriden Infection.

1873 CLEMENTI U. THIN.—Unters. üb. d. Putride Infection. Wien. Med. Jahrb.

1874 PANUM.—Virchow's Arch., Bd. 60.

FRISCH.—Exper. Studien üb. d. Verbreitung d. Fäulnissorganismen.

KEHRER.—Archiv. f. Exper. Pathol., Bd. I.

1876 HILLER.—Centralbl. f. Chirurgie.

NENCKI.—Ueber die Zersetzung der Gelatine und des Eiweisses bei der Fäulniss mit Pancreas.

1877 SALOMONSEN.—Die Fäulniss des Blutes.

1878 SELMI.—Chemische Ber., Bd. 6, 7, 12, Sulle Ptomaine ad Alcaloide Cadaverici.

KAUFMANN.—Zersetzung des Blutes durch Bacillus Subtilis. Journ. f. Prakt. Chemie, Bd. 17.

1879 HILLER.—Die Lehre von der Fäulniss.
1880 HUSEMANN.—Arch. d. Pharmac.
1881 KÖNIG.—Massenerkrankung von Menschen nach dem Genuss von Fleisch einer an Putrider Metritis Verendeten Kuh. Ber. üb d. Veterinärwesen im Königreich Sachsen.
TANRET.—Compt. Rend., T. 92.
BROUARDEL ET BOUTMY.—Compt. Rend., T. 92, p. 1056.
1882 HUSEMANN.—Arch. d. Pharmac.
SCHIFFER.—Arch. f. Anat. u. Physiol., Physiol. Abtheil.
BOCCI.—Centralbl. f. d. Med. Wiss.
GROEBNER.—Beiträge z. Kenntniss der Ptomaïne.
BERGMANN U. ANGERER.—Das Verhältniss der Fermentintoxication zur Septicämie. Würzburger Jubil. Festschr.
BOUCHARD.—Compt. Rend. de Biol.
NENCKI.—Journ. f. Pract. Chem., Bd. 26.
GAUTIER.—Compt. Rend., T. 94.
GAUTIER ET ETARD.—Compt. Rend , T. 94.
ROSENBACH.—Giebt es Verschiedene Arten von Fäulniss? Deut. Zeitschrift für Chir., XVI., S. 342.
ETARD ET OLIVIER.—De la Reduction des Sulfates par les Etres Vivants. Compt. Rend.
1883 HUSEMANN.—Arch d. Pharmac.
GUARESCHI ET MOSSO.—Arch. Ital. de Biolog.
BRIEGER.—Zur Kenntniss der Fäulnissalkaloide. Zeitschr. f. physiol. Chemie, Bd. 7.
1884 MAAS.—Ueber Fäulnissalkaloide des gekochten Fleisches und des Fischfleisches. Fortschr. d. Med., II., 729.
BRIEGER.—Ber. d. Deutsch. Chem. Ges., Bd. 17.
VANDEVELDE.—Les Ptomaïnes. Arch. de Biol. par van Beneden.
WILLGERODT.—Ueber Ptomaïne.

1884 BRIEGER.—Ueber Giftige Producte der Fäulnissbakterien. Berl. Klin. Woch., Nr. 14.
OTTO.—Anleitung zur Ausmittelung der Gifte.
1885 TAPPEINER.—Med. Centralbl.
BLUMBERG.—Experimenteller Beitrag zur Kenntniss der Putriden Intoxication. Virch. Arch., Bd. 100, S. 377.
OFFINGER.—Die Ptomaïne.
BRIEGER.—Ueber Ptomaïne.
BRIEGER.—Weitere Untersuchungen über Ptomaïne.
BACKLISCH.—Ber. d. Deutsch. Chem. Gesellsch., Bd. 18.
HAUSER.—Ueber Fäulnissbakterien.
1886 BRIEGER.—Ueber Ptomaïne. Berl. Klin. Woch.

(H) *ANTISEPTICS AND DISINFECTANTS.*

1875 EIDAM.—Einwirkung Versch. Temperaturen a. d. Entwicklung von Bacterium Termo. Cohn's Beitr. zur. Biol., Bd. I., Heft 3.
SCHRÖTER.—Cohn's Beitr. zur Biol. der Pflanzen, Bd. I., Heft 3.
1876 KOCH.—Cohn's Beitr. zur Biol. der Pflanzen, Bd. II., Heft 2.
BUCHHOLZ.—Ueber das Verhalten von Bakterien zu einigen Antiseptics.
1877 NÄGELI.—Die Niederen Pilze.
BUCHHOLZ.—Arch. f. Exp. Pathol., Bd. 7.
FRISCH.—Ueber den Einfluss nied. Temp. auf. d. Lebensfähigkeit der Bakterien. Sitzungsber. d. Wiener Akad., Bd. 75 u. 80.
TYNDALL.—Philos. Transact. of the Roy. Soc.
VALLIN.—Ann. d'Hyg.
1879 SOYKA.—Ber. d. Bayr. Akad. d. Wissensch.
SCHWARTZ.—Sitzungsber. d. Dorpater Naturf. Ges.
WERNICKE.—Diss. Dorpat.

1879 HABERKORN.—Das Verhalten von Harnbakterien gegen einige Antiseptica. Dissert. Dorpat.
WERNICH.—Die Aromatischen Fäulnissproducte in ihrer Einwirkung auf Spalt und Sprosspilze. Virchow's Archiv, Bd. 78.
1880 MERKE.—Virchow's Archiv, Bd. 81.
MÖRSCHELL.—Deut. Med. Woch.
LASSAR.—Deut. Med. Woch.
PASTEUR.—Ann. d'Hyg.
WERNICH.—Grundriss der Desinfectionslehre.
MEYER.—Ueb. d. Milchsäureferment u. sein Verhalten gegen Antiseptica.
TOUSSAINT.—Bull. de l'Acad.
1881 WOLFFHÜGEL.—Ueber den Werth der schwefligen Säure als Desinfectionsmittel. Mittheilg. a. d. Kais. Ges. Amt, Bd. I., S. 188.
WOLFFHÜGEL U. KNORRE.—Zu der verschiedenen Wirksamkeit von Carbolöl u. Carbolwasser. Mitth. a. d. Kaiserl. Ges. Amt, Bd. I., S. 352.
KOCH.—Ueber Desinfection. Mitth. a. d. Ges. Amt, Bd. I., S. 234.
DE LA CROIX.—Das Verhalten der Bakterien d. Fleischwassers gegen einige Antiseptica. Arch. f. Exp. Pathol., Bd. 13.
KOCH, GAFFKY U. LÖFFLER.—Versuche über die Verwerthbarkeit heisser Wasserdämpfe zu Desinfectionszwecken. Mitth. a. d. Kaiserl. Ges. Amt, Bd. I., S. 322.
KOCH U. WOLFFHÜGEL.—Unters. üb. die Desinfection mit heisser Luft. Mitth. a. d. Kaiserl. Ges. Amt, Bd. I., S. 301.
HUEPPE.—Ueber das Verhalten ungeformter Fermente gegen hohe Temperatur. Mittheilg. a. d. Kaiserl. Ges. Amt, Bd. I., S. 341.
1882 LEBEDEFF.—Desinfectionsversuche am Malignen Oedem. Arch. de Physiol. Norm. et Pathol.
W. CHEYNE.—Antiseptic Surgery.

1882 LEBEDEFF.—Arch. de Phys. Norm. et Pathol.
HUEPPE.—Ueber die Hitze als Desinfectionsmittel. Deut. Militärärztl. Zeitschr.
1883 FISCHER.—Unters. üb. d. Wirkung des Naphtalins. Berl. Klin. Woch.
PERRONCITO.—Sur la Tenacité de Vie du Virus Charbonneux. Arch. Ital. de Biol.
VALLIN.—Traité des Désinfectants et de la Désinfection.
MIQUEL.—Antiseptiques et Bactéries. Semaine Médicale.
CHAUVEAU.—Compt. Rend.
CHAMBERLAND ET ROUX.—Compt. Rend.
LAILLIER.—Du Gaz Acide Sulfureux. Ann. d'Hygiène.
ARLOING, CORNEVIN, ET THOMAS.—Lyon Méd.
VALLIN.—Les Nouvelles Etuves à Desinfection. Revue d'Hygiène.
LARRIVÉ.—L'Eau Oxygénée. Thèse de Paris.
MALY U. EMICH.—Antisept. Wirkung der Gallensäuren. Sitzungsber. d. Kais. Akad. d. Wiss. zu Wien. Jan.
SCHULTZ.—Die Antiseptischen Eigenschaften der Citronensäure. Deut. Med. Woch., Nr. 17.
1884 SCHILL U. FISCHER.—Ueber die Desinfection des Auswurfs der Phthisiker. Mitth. a. d. Kaiserl. Ges. Amt, Bd. II.
PLAUT.—Desinfection der Viehställe.
STEINMEYER.—Ueber Desinfectionslehre.
COLIN.—Compt. Rend., T. 99.
CHAIRY.—Action des Agents Chimiques sur les Bactéries du Genre Tyrothrix. Compt. Rend.
CHAUVEAU.—Compt. Rend.
MIGNET.—Annuaire de l'Observatoire de Montsouris.
SCHNETZLER.—Les Propriétés Antiseptiques de l'Acide Formique. Archiv. de Génève.

1884 ROSSBACH.—Einfluss des innerl. Naphthalingebrauchs auf die Harnfäulniss. Berl. Klin. Woch.
HOFFMANN.—Experimentelle Untersuchungen über die Wirkung der Ameisensäure. Diss. Greifswald.
FISCHER U. PROSKAUER.—Mitth. a. d. Kaiserl. Ges. Amt, Bd. II.
HEYDENREICH.—Sur la Stérilisation des Liquides au moyen de la Marmite de Papin. Compt. Rend., T. 98.
PICTET ET YOUNG.—De l'Action du Froid sur les Microbes. Compt. Rend., T. 98.
VALLIN.—Ann. d'Hygiène.
ROCHEFORT, HERSCHER.—Revue d'Hygiène.
DUJARDIN-BEAUMETZ.—Bull. de l'Acad. de Méd. de Paris.
MARIÉ-DAVY.—Revue d'Hygiène.
1885 M. WOLFF.—Zur Desinfectionsfrage. Centralbl. f. d. Med. Wiss., Nr. 11.
REINL.—Zur Theorie der Heilwirkung des Franzensbader Moores. Prager Med. Woch., Nr. 10 u. 11.
THOL.—Ueber d. Einfluss nicht aromat. organ. Säuren auf Fäulniss u. Gährung. Diss. Greifswald.
SCHULZ.—Die Ameisensäure als Antisepticum. Deut. Med. Woch., Nr. 24.
SCHEDE.—Die Antiseptische Wundbehandlung mit Sublimat. Sammlung Klin. Vorträge, Nr. 25.
KÖNIG.—Sublimatdämpfe. Chirurg. Centralbl.
FRANK.—Ueber Desinfection von Abtrittsgruben.
GÄRTNER U. PLAGGE.—Deut. Med. Woch.
1886 HANDFORD.—Brit. Med. Journ.
KOCH U. GAFFKY.—Arbeit. a. d. K. Gesundh. Amt.
WINTER BLYTH.—Studies of Disinfectants by New Methods. Proc. Roy. Soc.

(I) *IMMUNITY.*

1876 OEMLER.—Arch. f. Wiss. u. Pract. Thierheilk.

1879 OLLIVE.—Sur la Résistance des Moutons de la Race Berberine à l'Inoculation du Charbon. Compt. Rend., T. 89.

CHAUVEAU.—De la Prédisposition et de l'Immunité Pathologique. Compt. Rend., T. 89.

1880 PASTEUR.—Bull. de l'Acad. de Méd. and Gaz. Méd. de Paris, Nr. 18.

SEMMER U. KRAJEWSKI.—Centralbl. f. d. Med. Wiss.

TOUSSAINT.—Bull. de l'Acad. de Méd.; and Compt. Rend.

1881 TOUSSAINT.—Compt. Rend.

LOEFFLER.—Zur Immunitätsfrage. Mitth. a. d. Ges. Amt, Bd. I.

SEMMER.—Virchow's Arch., Bd. 83.

GRAWITZ.—Die Theorie der Schutzimpfung. Virchow's Arch., Bd. 84.

TOUSSAINT.—Gazette Médicale de Paris, Nr. 32.

OEMLER.—Arch. f. Wiss. u. Pract. Thierheilk.

1882 RÓSZAHEGYI.—Pester Med.-Chir. Presse.

KOCH.—Ueber die Milzbrandimpfung.

1883 FRANK.—Jahresber. d. K. Thierarzneischule in München.

ARLOING, CORNEVIN ET THOMAS.—Du Charbon Bactérien; Pathogénie et Inoculations Préventives.

CHAUVEAU.—Compt. Rend., T. 96, Nr. 9.

CHAUVEAU.—Compt. Rend., T. 96, Nr. 10.

CHAUVEAU.—Du Rôle de l'Oxygène de l'Air dans l'Attenuation quasi-instantanée des Cultures Virulentes par l'Action de la Chaleur. Compt. Rend., T. 96, Nr. 11.

1883 PASTEUR.—Sur la Vaccination Charbonneuse. Compt. Rend.

PASTEUR.—La Vaccination Charbonneuse.

PASTEUR.—Réponse au Doct. Koch. Revue Scientifique.

PASTEUR.—Bull. de l'Acad. de Méd.

BÜCHNER.—Eine neue Theorie über Erzielung v. Immunität gegen Infectionskrankheiten.

PERRONCITO.—Sull' Attenuazione del Virus Carbonchioso. Atti R. Acc. d. Lincei.

CHAMBERLAND ET ROUX.—Compt. Rend., T. 96, Nr. 15.

CHAMBERLAND.—Le Charbon et la Vaccination Charbonneuse d'après les Travaux Récents de M. Pasteur.

MASSÉ.—Des Inoculations Préventives dans les Maladies Virulentes.

1884 FELTZ.—De la Durée de l'Immunité Vaccinale Anticharbonneuse chez le Lapin. Compt. Rend., T. 99, p. 246.

CHAUVEAU.—De l'Attenuation des Cultures Virulentes par l'Oxigène Comprimé. Gaz. Hebdom. de Méd. et de Chir., 22.

KOCH, GAFFKY U. LOEFFLER.—Exper. Studien über d. Künstl. Abschwächung der Milzbrandbacillen. Mitth. a. d. Ges. Amt, Bd. II.

BLAZEKOVIC.—Zur Präventiv-Inoculation Pasteur's. Oesterr. Monatschr. f. Thierheilk.

PÜTZ.—Vorträge f. Thierärzte, Ser. 7, Heft 1.

1885 HESS.—Vorl. Mitth. ü. die Schutzimpfung gegen Milzbrand im Kanton Bern nach der Methode Chauveau. Schweiz. Arch. f. Thierheilk., Bd. 27.

STREBEL.—Zur Rauschbrandimpfung. Schweiz. Arch. f. Thierheilk.

BOULEY.—L'Inoculation Préventive de la Fièvre Jaune. Compt. Rend., T. 100.

(J) *BACTERIA ASSOCIATED WITH DISEASES IN MAN AND ANIMALS.*

(I.) Actinomycosis.

1877 BOLLINGER.—Centralbl. f. d. Med. Wiss.
1878 ISRAËL.—Virchow's Arch., Bd. 74.
1879 ISRAËL.—Virchow's Arch., Bd. 78.
1881 PONFICK.—Die Actinomykose. Berlin.
JOHNE.—Deutsche Zeitschr. f. Thiermed.
1882 HINK.—Centralbl. f. d. Med. Wiss.
PFLUG.—Centralbl. f. d. Med. Wiss.
GANNET.—Boston Med. and Surg. Journ.
1883 PUSCH.—Arch. f. Wiss. u. Pr. Thierheilk.
BANG.—Tidskrift far Veterinaerer.
ZEMANN.—Wien. Med. Jahrb., S. 477.
FLEMING.—Actinomycosis.
1884 ISRAËL.—Virchow's Arch., Bd. 96.
MITTELDORPF.—Deut. Med. Woch.
KARSTEN.—Deut. Med. Woch.
CHIARI.—Prager Med. Woch., Nr. 10.
TREVES.—Lancet.
FIRKET.—Rev. de Méd.
1885 ISRAËL.—Kenntniss der Actinomykose des Menschen. Klinische Beiträge.
BOSTRÖM.—Verh. d. Congr. f. Inn. Med. Wiesbaden.
BAUMGARTEN.—Berl. Klin. Woch.
JOHNE.—Bericht ü. d. Veter.-Wesen i. K. Sachsen.
MURPHY.—New York Med. Journ.
PONFICK.—Breslauer Aerztl. Zeitschr.
SOLTMANN.—Breslauer Aerztl. Zeitschr.
MAGNUSSEN.—Beiträge zur Diagnostik u. Casuistik der Actinomykose. Diss. Kiel.
1886 HERTWIG.—Archiv. f. Wiss. u. Prakt. Thierheilk.
O'NEILL.—Lancet.
ACLAND.—Brit. Med. Journ. and Trans. Path. Soc.
ROSER.—Deut. Med. Woch.
ISRAËL.—Archiv f. Klin. Chir.

(II.) Acute Yellow Atrophy

1875 EPPINGER.—Prag. Viertelj.
1882 HLAVA.—Prag. Med. Wochenschr.
BALZER.—Archiv de Phys. Norm. et. Path.

(III.) Anthrax.

1855 POLLENDER.—Vierteljahrschr. f. Ger. Med., Bd. 8.
1857 BRAUELL.—Virchow's Arch., Bd. 11.
1858 BRAUELL.—Virchow's Arch., Bd. 14.
1863 DAVAINE.—Compt. Rend. Paris. T. 57. Ibid., T. 59.
1865 DAVAINE.—Compt. Rend. Paris. T. 60.
1866 DAVAINE.—Compt. Rend. Paris. T. 61.
1872 BOLLINGER.—Centralbl. f. d. Med. Wiss., Bd. 10.
1873 COLIN.—Bull. Acad. de Méd., Paris. T. 2.
DAVAINE.—Compt. Rend. Paris. T. 77.
1876 KOCH.—Beiträge zur Biologie der Pflanzen, Bd. II., Heft 2.
1877 PASTEUR.—Compt. Rend. Paris. T. 84.
TOUSSAINT.—Compt. Rend. T. 85.
DAVAINE.—Rec. de Méd. Vét., T. 4.
DAVAINE.—Compt. Rend.
BERT.—Compt. Rend. Soc. de Biol., T. 4.
PASTEUR.—Bull. Acad. de Méd.
1878 COLIN.—Bull. Acad. de Méd. Paris. T. 7.
TOUSSAINT.—Compt. Rend. Paris.
OEMLER.—Archiv f. Wiss. u. Pract. Thierheilk., Bd. 4.
EWART.—Quart. Journ. of Microsc. Sc.
BERT.—Compt. Rend. Soc. de Biol., T. 5.
KOCH.—Wundinfectionskrankheiten.
1879 SCHMIDT.—Milzbrand bei Wildschweinen. Deut. Zeitschr. f. Thiermed. u. Vergl. Pathol.

1879 TOUSSAINT.—Recherches Expérimentales sur la Maladie Charbonneuse.
BERT.—Compt. Rend. Soc. de Biol., T. 6.
GREENFIELD.—Quart. Journ. Micr. Sc. London.
OEMLER.—Archiv. f. Wiss. u. Pract. Thierheilk.
COLIN.—Bull. Acad. de Méd. Paris. T. 8.
PASTEUR.—Bull. Acad. de Méd. Paris.
1880 PASTEUR.—Compt. Rend. Paris. T. 90 and 91.
CHAUVEAU.—Compt. Rend. T. 90 and 91.
TOUSSAINT.—Compt. Rend.
COLIN.—Bull. Acad. de Méd. Paris. T. 9.
PASTEUR.—Bull. Acad. de Méd. Paris.
BOULEY.—Bull. Acad. de Méd. Paris. T. 9.
WACHENHEIM.—Etude Expérimentelle sur la Septicité et la Virulence du Sang Charbonneux.
BÜCHNER.—Ueber die Exper. Erzeugung des Milzbrandcontagiums aus den Heupilzen.
BÜCHNER.—Versuche über die Entstehung des Milzbrands durch Einathmung. Sitzungsber. d. K. Bayer. Akad. d. Wissensch.
FOKKER.—Centralbl. f. d. Med. Wissensch. Bd. 18.
SEMMER.—Centralbl. f. d. Med. Wiss., Bd. 18.
OEMLER.—Archiv f. Wissensch. u. Pract. Thierheilk.
SZPILMAN.—Zeitschr. f. Physiol. Chemie. Strassburg. Bd. 4.
GREENFIELD.—Proc. Roy. Soc. London.
1881 PASTEUR.—Compt. Rend. Paris. T. 92.
CHAUVEAU.—Compt. Rend., T. 92.
BOULEY.—Compt. Rend. T. 92. Ibid., T. 93.
COLIN.—Bull. Acad. de Méd., T. 10.
BOULEY.—Bull. Acad. de Méd., T. 10.
RODET.—Contribution a l'Etude Expérimentelle du Charbon Bactéridien.
KOCH.—Mitth. aus. d. Ges. Amt, Bd. I.
FOKKER.—Centralbl. f. d. Med. Wiss.
BÜCHNER.—Vorträge im Aerztl. Verein zu München.

1881 HUBER.—Deut. Med. Woch., Bd. 7.
KLEIN.—Rep. of the Medical Officer of the Local Govt. Board.
STERNBERG.—Am. Monthly Micr. Journ.
1882 CHAUVEAU.—Compt. Rend. Paris. T. 94.
RODET.—Compt. Rend., T. 94.
PASTEUR.—Compt. Rend. Paris. T. 95.
FELTZ.—Compt. Rend., T. 95.
FOKKER.—Virchow's Archiv, Bd. 88.
ARCHANGELSKI.—Centralbl. f. d. Med. Wiss.
SEMMER.—Der Milzbrand und das Milzbrandcontagium.
ESSER U. SCHÜTZ.—Zur Casuistik des Milzbrands. Mitth. a. K. Preuss. Amtl. Vet. Sanitätsbericht.
1883 CHAUVEAU.—Compt. Rend. Paris. T. 96.
ARCHANGELSKI.—Centralbl. f. d. Med. Wiss.
ROLOFF.—Ueber die Milzbrandimpfung und d. Entwicklung d. Milzbrandbakterien. Archiv f. Wissensch u. Pract. Thierheilk., Bd. 9.
ROLOFF.—Der Milzbrand. Berlin.
BÜCHNER.—Die Umwandlung der Milzbrandbakterien in unschädliche Bakterien. Virchow's Arch., Bd. 91.
TOEPPER.—Die Neueren Erfahrungen über d. Aetiologie d. Milzbrands.
KLEIN.—Quart. Journ. Micr. Sc.
DOWDESWELL.—Rep. Med. Off. Local Gov. Board.
1884 BLEULER.—Milzbrand beim Menschen. Correspondenzbl. d. Schweiz. Aerzte.
SCHRAKAMP.—Zur Aetiologie des Milzbrandes. Archiv f. Hygiene, Bd. 2.
v. CHELCHOWSKY.—Zur Charakteristik des Milzbrandvirus. Der Thierarzt.
SEMMER.—Centralbl. f. d. Med. Wiss., Bd. 22.
PRAZMOWSKI.—Acad. d. Wissensch. in Krakau.
PRAZMOWSKI.—Biol. Centralbl., Bd. 4.

1885 BOLLINGER.—Zur Aetiologie des Milzbrands. Sitzungsber. d. Ges. f. Morphol. Physiol. zu München.

KITT.—Sitzungsb. d. Ges. f. Morphol. u. Physiol. zu München.

FRIEDRICH.—Zur Aetiologie des Milzbrands.

OSOL.—Experiment. Untersuch. ü. das Anthraxgift. Inaug. Diss. Dorpat.

1886 W. KOCH.—Milzbrand und Rauschbrand. Stuttgart.

HOFFA.—Die Natur des Milzbrandgiftes. Wiesbaden.

v. FODOR.—Deut. Med. Woch.

JOHNE.—Ber. ü. d. Veter.Wesen. i. K. Sachsen.

BOLLINGER.—Arbeit. a. d. Patholog. Inst. zu München.

FRANK.—Zeitschr. f. Hygiene.

(IV.) Cattle Plague.

1883 SEMMER U. ARCHANGELSKI.—Ueber das Rinderpestcontagium und dessen Mitigation. Centralbl. f. d. Med. Wiss.

(V.) Cerebro-Spinal Meningitis.

1883 LEYDEN.—Die Mikrokokken der Cerebrospinal-Meningitis. Centralbl. f. Klin. Med., Nr. 10.

1885 LEICHTENSTERN.—Deut. Med. Woch., Nr. 23 u. 31.

(VI.) Chicken-Cholera.

1877 IOANNÈS ET MÉGNIN.—Journ. d'Acclimatation.

1878 SEMMER.—Hühnerpest. Deut. Zeitschr. f. Thiermed. u. Vergl. Path.

1879 PERRONCITO.—Ueber das Epizootische Typhoïd der Hühner. Arch. f. Wiss. u. Prakt. Thierheilk.
1880 PASTEUR.—Sur le Choléra des Poules. Compt. Rend., T. 90.
1882 ZÜRN.—Die Krankheiten des Hausgeflügels.
CORNIL.—Arch. de Physiol., Bd. 10.
1883 BARTHÉLÉMY.—De l'Incubation des Œufs d'une Poule atteinte du Choléra des Poules. Compt. Rend., T. 96., No, 18.
BABÈS.—Arch. de Physiol.
BABÈS.—Compt. Rend. de l'Acad. d. Sc.
1884 PETRI.—Centralbl. f. d. Med. Wiss.
1885 KITT.—Mitth. über die Typhoïdseuche des Geflügels. Allg. Deut. Geflügelzeitung.

(VII.) Cholera.

1883 STRAUSS, ROUX, THUILLIER ET NOCARD.—Compt. Rend. Soc. de Biol., T. 4.
1884 FINKLER U. PRIOR.—Ueber den Bacillus der Cholera Nostras und seine Cultur. Naturforscherversammlung Magdeburg.
PFEIFFER.—Der bisherige Verlauf der Cholera in Thüringen, etc. Corresp.-Bl. des Allgem. Aerztl. Ver. in Thüringen, Nr. 9.
KOCH.—Ueber die Cholerabakterien. Deut. Med. Woch.
EMMERICH.—Die Cholera in Neapel. Deut. Med. Woch., Nr. 50.
FINKLER U. PRIOR.—Unters. über Cholera Nostras. Deut. Med. Woch., Nr. 36.
DOYEN.—Mikro-organismen in Leber und Niere von Choleraleichen. Soc. de Biol. de Paris. Dec. 13.
BÜCHNER.—Ueber Cholerabacillen. Münchn. Aerztl. Intelligenzbl., S. 549.

1884 BIANCHI.—Lancet.
MACNAMARA.—Brit. Med. Journ.
HUNTER.—Brit. Med. Journal.
CARTER.—Lancet.
CAMERON.—Brit. Med. Journ.
BOCHEFONTAINE.—Expér. pour servir à l'Etude des Phénomènes déterminés chez l'Homme par l'Ingestion Stomacale du Liquide Diarrhéïque du Choléra. Compt. Rend.
LEWIS.—Med. Times and Gazette. September 20th.
1885 JOHNE.—Einiges über die sogen. Choleracurse im. K. Ges. Amt. Deutsche Zeitschr. f. Thiermed., Bd. XI.
FINKLER U. PRIOR.—Forschungen über Cholerabakterien. Ergänzungshefte zum Centralbl. f Allg. Gesundheitspflege, Bd. I., Heft 5 u. 6.
WATSON CHEYNE.—Brit. Med. Journ.
BABÈS.—Virchow's Archiv.
FERRAN—Sur l'Action Pathogène et Prophylactique du Bacillus-virgule. Compt. Rend., T. 100, p. 959.
HÉRICOURT.—Sur la Nature Indifférente des Bacilles-virgules. Compt. Rend.
VILLERS.—Sur la Formation des Ptomaïnes dans le Choléra. Ibid., p. 91.
FLÜGGE.—Kritik der Emmerich'schen Untersuchungen über Cholera. Deut. Med. Woch., Nr. 2.
PFEIFFER.—Ueber die Cholera in Paris. Deut. Med. Woch., Nr. 2.
DENEKE.—Ueber eine Neue den Choleraspirillen ähnliche Spaltpilzart. Deut. Med. Woch., Nr. 3.
MILLER.—Kommaförmiger Bacillus aus der Mundhöhle. Deut. Med. Woch., Nr. 9.
KLEBS.—Ueber Cholera Asiatica.

1885 SCHOTTELIUS.—Zum Mikrosk. Nachw. v. Cholera-bacillen in Dejectionen. Deut. Med. Woch., Nr. 14.

DRASCHE.—Allg. Wien. Med. Zeit.

VAN ERMENGEM.—Die Ferran'schen Impfungen. Deut. Med. Woch., Nr. 29.

GIBIER ET VAN ERMENGEM. Rech. Expér. sur le Choléra. Compt. Rend., T. 101.

VAN ERMENGEM.—Recherches sur le Microbe du Choléra Asiatique.

NICATI AND RIETSCH.—Arch. de Physiol.

NICATI AND RIETSCH.—Revue d'Hygiène.

NICATI AND RIETSCH.—Revue de Médecin, T. 5.

BRUNETTI.—Fatti Considerazioni Conclusioni sul Coléra.

KLEIN.—Brit. Med. Journ. and Proc. Roy. Soc. London, No. 38.

KLEIN and GIBBES.—An Inquiry into the Etiology of Asiatic Cholera. Bluebook.

BÜCHNER.—Archiv. f. Hygiène.

BÜCHNER U. EMMERICH.—Münch. Med. Woch.

CROOKSHANK.—Remarks on the Comma Bacillus of Koch. Lancet.

1886 KOCH.—Etiology of Cholera. Berlin Cholera Conference. Translated by Laycock, in Microparasites and Disease (New Syd. Soc.)

PETTENKOFER (abstracted by Koplik).—Lancet.

CANTANI.—Deut. Med. Woch.

WEISSER.—Zeitschrift f. Hygiene.

BITTER.—Archiv f. Hygiene.

PFEIFFER.—Deut. Med. Woch.

KLEIN.—Bacteria of Asiatic Cholera. Practitioner.

WEISSER and FRANK.—Zeitschr. f. Hygiene.

(VIII.) Dental Caries.

1867 LEBER U. ROTTENSTEIN.—Unters. über Caries der Zähne. Berlin.
1880 ARNDT.—Beob. an Spirochæta Denticola. Arch. f. Pathol. Anat., Physiol. u. Klin. Med., Bd. 79.
1882 MILLER.—Der Einfluss der Mikroorganismen auf die Caries der Zähne. Arch. f. Exp. Path., XVI.
1883 MILLER.—Ueber einen Zahnspaltpilz Leptothrix Gigantea. Ber. d. Deutsch. Bot. Ges., Heft 5.
1884 MILLER.—Deutsche Med. Woch., Nr. 25 u. 36.
MILLER.—Correspdzbl. f. Zahnärzte, Bd. 13.
ROSENBACH.—Mikroorganismen bei den Wundinfectionskrankheiten, S. 77.

(IX.) Diphtheria.

1867 BUHL.—Micrococci of Diphtheria. Zeitschr. für Biol.
1868 HUETER U. TOMMASI.—Centralbl. f. d. Med. Wissensch.
1871 OERTEL.—Deut. Arch. f. Klin. Med., Bd. 8.
1872 LETZERICH.—Virch. Arch., Bd. 55.
BIRCH-HIRSCHFELD.—Archiv für Heilkunde.
1873 EBERTH. — Der Diphtheritische Process. Med. Centralbl., XI., Nr. 8.
1874 LETZERICH.—Mikrochemische Erscheinungen des Diphtheritispilzes. Berl. Klin. Woch., XI.
1875 KLEBS.—Arch. f. Exp. Pathol., Bd. 4.
1876 LETZERICH.—Virch. Arch., Bd. 68.
1878 ZAHN.—Beiträge zur Pathol. u. Histol. der Diphtherie.
1879 FRIEDBERGER.—Ueber Croup u. Diphtheritis beim Hausgeflügel. Deut. Zeitschr. für Thiermed. u. Vergl. Pathol.

1879 NICATI.—Compt. Rend., T. 88.
1880 NEUMAYER.—Neue Thesen zur Diphtheritisfrage.
1881 EVERETT.—Med. and Surg. Reporter.
TALAMON.—Bull. de la Soc. Anat. de Paris. T. 56.
CORNIL.—Arch. de Physiol.
OERTEL.—Zur Aetiologie der Infectionskrankheiten.
FORSTER.—Wien. Med. Woch.
LUMNER.—Aerztl. Int. Bl., No. 31.
1882 WOOD AND FORMAD.—Bull. Nat. Board of Health, Wash. and Med. Times and Gazette.
SALISBURY.—Gaillard's Med. Journ. New York.
GERHARDT AND KLEBS.—Verhandlung des Congresses für Innere Med.
1883 FÜRBRINGER.—Virch. Arch., Bd. 91.
HEUBNER.—Die Experimentelle Diphtherie.
GERHARDT U. KLEBS.—Verhandl. d. Congresses f. Inn. Med.
FRANCOTTE.—La Diphthérie.
1884 EMMERICH.—Deut. Med. Woch.
EMMERICH.—Compt. Rendus et Mémoires du V. Congrès Internat. d'Hygiène.
RIVOLTA.—Die Diphtherie der Hühner im Vergleich zu der des Menschen. Giornale de Anat. Fisiol. e Patol. delli Anim.
LOEFFLER.—Mittheil. a. d. Kais. Ges. Amt, Bd. II.
1886 LOEFFLER.—Microparasites and Disease [New Syd. Soc.].

(X.) Erysipelas.

1868 HÜTER.—Med. Centralbl., Nr. 34.
1873 RAYNAUD.—Union Méd.
ORTH.—Archiv. f. Exper. Pathol. u. Pharmacol., Bd. I.

1874 RECKLINGHAUSEN U. LANKOWSKI.—Ueber Erysipelas. Virchow's Arch., Bd. 60.
LUKOMSKY.—Virchow's Archiv, Bd. 60.
1875 TROSIER.—Bull. Soc. Anat. de Paris.
BAADER.—Zur Aetiologie des Erysipels. Schweiz. Naturf. Gesellsch.
KLEBS.—Archiv f. Exper. Pathol. u. Pharmacol., Bd. 4.
1878 TILLMANNS.—Verhandl. d. Deutsch. Ges. f. Chirurgie.
1879 TILLMANS. — Experimentelle u. Anatomische Unters. etc. Archiv f. Klin. Chirurgie, Bd. 23.
1880 WOLFF.—Virchow's Arch., Bd. 81.
1881 DUPEYRAT.—Recherches Cliniques et Expérimentales sur la Pathogénie de l'Erysipèle.
FEHLEISEN.—Ueber den Erysipelaspilz. Würzburger Phys. Med. Ges.
1882 FEHLEISEN.—Deut. Zeitschr. f. Chir., Bd. 16.
1883 FEHLEISEN.—Die Aetiologie des Erysipels. Berlin.
1884 JANICKE U. NEISSER.—Exitus Lethalis nach Erysipelas. Centralbl. f. Chir., Nr. 25.
RHEINER.—Beitr. z. Path. Anat. des Erysipels bei Gelegenheit der Typhusepid. in Zürich. Virchow's Arch., Bd. 100, Heft 2.
1885 NEPVEN.—Des Bactéries dans l'Erysipèle.
DENUCE.—Etude sur la Pathogénie et l'Anatomie pathologique de l'Erysipèle.

(XI.) Endocarditis.

1875 KOESTER.—Virchow's Arch., Bd. 72.
1876 BIRCH-HIRSCHFELD U. GERBER.—Archiv d. Heilkunde.
1877 WEDEL.—Berl. Klin. Wochenschr.

1878 KLEBS.—Archiv f. Exper. Pathol., Bd. 9.
O. ROSENBACH.—Ueber Artificielle Herzklappenfehler. Archiv für Exper. Pathol., Bd. 9.
1880 HAMBURG.—Ueber Acute Endocarditis. Berlin. Inaug.-Diss.
GOODHART.—Trans. Path. Soc., Vol. XXXI.
1881 OBERBECK.—Casuistische Beiträge zur Lehre von der Endocarditis Ulcerosa. Inaug.-Diss.
LEYDEN.—Zeitschr. f. Klin. Med.
WEIGERT.—Virchow's Arch., Bd. 84.
KOCH.—Mittheil. a. d. Kais. Ges. Amt, Bd. I.
OSLER.—Trans. Int. Med. Congress.
1882 WILKS.—Brit. Med. Journ.
1883 KUNDRAT.—Sitz.-Ber. d. Kais. Acad. d. Wissensch. zu Wien.
1884 BRAMWELL.—Diseases of the Heart.
BRISTOWE.—Brit. Med. Journ.
GIBBES.—Brit. Med. Journ.
1885 WYSSOKOWITSCH. — Centralbl. f. d. Med. Wissensch., Nr. 33.
COUPLAND.—Brit. Med. Journ.
ORTH, J.—Versammlung Deutscher Naturf. zu Strasburg.
NOCARD.—Recueil de Méd. Vét.
OSLER.—Brit. Med. Journ.
WEICHSELBAUM.—Wien. Med. Woch.
1886 RIBBERT.—Fortsch. d. Med.

(XII.) Glanders.

1882 LÖFFLER U. SCHÜTZ.—Ueber den Rotzpilz. Deut. Med. Woch., Nr. 52.
BOUCHARD, CAPITAN ET CHARRIN. — Bull. de l'Acad. d. Sc., Nr. 51.
1883 ISRAËL.—Berl. Klin. Woch., Nr. 11.
WASSILIEFF.—Deut. Med. Woch., Nr. 11.

1883 MOLKENTIN.—Zur Sicherstellung der Diagnose von Rotz. Inaug.-Diss.
STRUCK.—Deut. Med. Woch., Nos. 51 u. 52.
VULPIAN AND BOULEY.—Bull. de l'Acad. de Méd.
FRÖHNER. — Rotzige Elephantiasis des Kopfes beim Pferde. Rep. d. Thierheilk.
1884 KITT.—Versuche über d. Züchtung des Rotzpilzes. Jahresber. d. München. Thierarzneisch.
GRÜNWALD.—Zur Differentialdiagnose des Rotzes. Oesterr. Monatsschr. für Thierheilk., Nr. 4.
WEICHSELBAUM.—Zur Aetiologie der Rotzkrankheit des Menschen. Wiener Med. Woch., 21—24.
1886 LOEFFLER.—Arbeit. a. d. K. Gesundh. Amt.

(XIII.) Gonorrhœa.

1879 NEISSER.—Centralbl. f. d. Med. Wiss., Nr. 28.
RETER.—Centralbl. f. d. Med. Wiss.
1880 BÜCKER.—Ueber Polyarthritis Gonorrhoica. Diss.
BOKAI.—Ueber das Contagium der Acuten Blennorrhœ. Allgem. Med. Centralzeitung, Nr. 74.
BOKAI AND FINKELSTEIN.—Prager Med. Chir. Presse.
WEISS.—Le Microbe du Pus Blennorrhagique.
1881 AUFRECHT.—Pathologische Mittheilungen.
HAAB. — Der Mikrokokkus der Blennorrhœa Neonator.
HIRSCHBERG U. KRAUSE.—Zur Pathologie der Ansteckenden Augenkrankheiten. Centralbl. f. Pract. Augenheilk.
1882 MARTIN.—Rech. sur les Inflamm. Métast. à la suite de la Gonorrhée.
KRAUSE. — Die Mikrokokken der Blenorrhœa Neonator.
LEISTIKOW.—Ueber Bakterien bei den Venerischen Krankheiten. Charité-Annalen, 7 Jahrg., S. 750

1882 BOCKHART.—Sitzungsbericht d. Phys. Med. Ges. zu Würzburg.

1883 BOCKHART.—Beitrag z. Aetiologie und Pathol. des Harnröhrentrippers. Viertelj. f. Dermatol. und Syph.

ARNING.—Gonokokken bei Bartolinitis. Viertelj. f. Dermatol. u. Syph., S. 371.

ESCHBAUM.—Beitr. zur Aetiologie der Gonorrh. Secrete. Deut. Med. Woch., S. 187.

NEWBERRY.—Maryland Med. Journ.

CAMPONA.—Italia Medica.

AUFRECHT.—Mikrokokken i. d. Inneren Organen bei Nabelvenenentzündung Neugeborener. Centalbl. f. d. Med. Wiss., Nr. 16.

PETRONE.—Sulla Natura dell'Artrite Blenorragica. Rivista Clin., No. 2.

1884 CHAMERON.—Progrès Medical, 43.

STERNBERG.—Med. News, Vol. 45, Nr. 16.

KAMMERER.—Ueber Gonorrh. Gelenkentzündung. Centralbl. f. Chirurgie, Nr. 4.

WELANDER.—Gaz. Med. de Paris.

KRONER.—Zur Aetiol. der Ophthalmoblennorrhœa Neonator. Naturforschervers. in Magdeburg, Arch. f. Gyn., XXV., S. 109.

SANGER. — Ueber Gonorrh. Erkrankung der Uterusadnexe. Naturforschervers. in Magdeburg. Ibid., S. 126.

OPPENHEIMER. Naturforschervers. in Magdeburg. Ibid., S. 51.

1885 FRÄNKEL.—Deut. Med. Woch., S. 22.

BUMM.—Der Mikroorganismus der Gonorrhoischen Schleimhauterkrankungen.

LUNDSTRÖM.—Studier öfver Gonokokkus. Diss. Helsingfors.

E. FRÄNKEL.—Mikrokokken bei Colpitis. Deut. Med. Woch., Nr. 2.

1886 SMIRNOFF.—Vrach.

(XIV.) Hydrophobia.

1881 COLIN.—Bull. Acad. de Méd. Paris, T. 10.
DOLÉRIS.—Gaz. Méd. de Paris, T. 3. Tribune Méd. Paris, T. 14.
PASTEUR.—Compt. Rend.
1882 PASTEUR, CHAMBERLAND, ROUX ET THUILLER. —Compt. Rend.
BERT.—Compt. Rend.
1883 GIBIER.—Compt. Rend.
1884 PASTEUR.—Nouvelle Communication sur la Rage. Ann. de Méd. Vétérin.
PERCHERON.—La Rage et les Expériences de M. Pasteur.
PASTEUR.—Compt. Rend.
FOL.—Acad. des Sciences.
1886 BABÈS.—In Les Bactéries.
DOWDESWELL.—Journ. Roy. Micro. Soc. and Lancet.
DOLAN.—Hydrophobia. M. Pasteur and his Methods.
PASTEUR.—Compt. Rend.
VIGNAL.—Brit. Med. Journ.
LANKESTER.—Nineteenth Century, No. 114.
KERR.—Brit. Med. Journ.
BAUER.—Münch. Med. Woch.
FRISCH.—Wien. Med. Woch.
HIME.—Experimental Researches concerning Pasteur's Prophylactic. Lancet.

(XV.) Leprosy.

1879 NEISSER.—Breslauer Aerztl. Zeitschr.
NEISSER.—Jahresber. d. Schles. Ges. für Vaterl. Cultur.

1880 HANSEN.—Virchow's Archiv.
GAUCHER ET HILLAIRET.—Progrès Méd.
1881 NEISSER.—Virchow's Archiv, Bd. 84.
CORNIL ET SUCHARD.—Ann. de Dermat. et Syph.
VOSSIUS.—Uebertragungsversuche von Lepra auf Kaninchen. Ber. über d. Ophthalmologencongress in Heidelberg.
JOHN HILLS.—On Leprosy in British Guiana.
1882 KÖBNER.—Uebertragungsversuche von Lepra auf Thiere. Virchow's Arch.
KÖBNER.—Virchow's Arch.
HAUSEN, ARMAUER.—Virchow's Arch., Bd. 90.
1883 DAMSCH.—Uebertragungsversuche von Lepra auf Thiere. Virch. Arch., Bd. 92. Centralbl. f. d. Med. Wissensch., Bd. 21.
KAPOSI.—Wiener Med. Woch.
HILLIS.—Trans. Path. Soc.
BABÈS.—Etude Comparative des Bactéries de la Lèpre et de la Tuberculose. Compt. Rend.
MORETTI.—Il Primo Caso di Lebbra nelle Marcho Confermato dalla Presenza del Bacillus Lepræ.
MÜLLER.—Deut. Archiv f. Klin. Med., Bd. 34.
1884 VIDAL.—La Lèpre et son Traitement.
ARNING.—Ueber das Vorkommen des Bacillus Lepræ bei Lepra Anæsthetica s. Nervorum. Virchow's Archiv, Bd. 97.
THIN.—Brit. Med. Journal.
1885 VIRCHOW.—Berl. Klin. Woch., Nr. 12.
UNNA.—Ueber Leprabacillen. Deut. Med. Woch., Nr. 32.
STEVEN.—Brit. Med. Journ.
1886 TOUTON.—Fortsch. d. Med.
UNNA.—Deut. Med. Woch.
NEISSER.—Virchow's Archiv.
UNNA U. LUTZ.—Dermatologische Studien, Heft I.
MELCHER U. ORTMANN.—Berl. Klin. Woch.

(XVI.) Malaria.

1879 KLEBS U. TOMMASI-CRUDELI. — Arch. f. Exp. Pathol., Bd. 2.

1880 TOMMASI-CRUDELI.—Der Bacillus Malariæ im Erdboden von Selinunte u. Campobello. Arch. f. Exp. Pathol., Bd. 12.

CUBONI AND MARCHIAFAVA.—Neue Studien üb. d. Natur der Malaria. Arch. f. Exp. Pathol., Bd. 13.

1881 STERNBERG. — Bull. Nat. Board of Health. Washington.

CUBONI AND MARCHIAFAVA. — Atti della R. Acad. dei Lincei.

LAVERAN.—Compt. Rend.

1882 MARCHAND.—Zur Aetiologie der Malaria. Virch. Archiv, Bd. 88.

ZIEHL.—Deutsch. Med. Woch., Nr. 48.

ROSZAHEGYI.—Von der Ursache des Wechselfiebers. Biol. Centralbl., Bd. 2.

TOMMASI-CRUDELI.—Die Malaria von Rom.

RICHARD.—Bestätigung der Laveranschen Beob. Compt. Rend., No. 8.

LAVERAN.—Les Parasites du Sang dans l'Impaludisme. Compt. Rend., No. 17.

BARDELS.—Gaz. des Hôpit.

1883 TORELLI.—La Malaria in Italia.

MAUREI.—L'Etiologie et la Nature du Paludisme. Ann. d'Hygiène.

1884 KOTELMANN.—Der Bacillus Malariæ im Alterthum. Virchow's Arch., Bd. 97.

GERHARDT.—Zeitschr. f. Klin. Med., Bd. 7.

SEHLEN.—Fortschr. d. Med., Bd. II.

LEONI.—Gazetta Medica di Roma.

MARCHIAFAVA AND CELLI. — Atti della R. Academia dei Lincei.

1884 MARIOTTI E CIARROCCHI.—Lo Sperimentale, T. 54.

TOMMASI-CRUDELI.—La Production Naturelle de la Malaria. Conférence faite à la 8. Sess. du Congrès Intern. Méd. à Copenhague.

LAVERAN.—Traité des Fièvres Palustres.

1885 MARCHIAFAVA' U. CELLI.—Neue Untersuchungen über die Malariainfection. Fortschr. d. Med., Bd. 3.

1886 CAMILLO GOLGI.—Archivio per le Scienze Mediche.

(XVII.) Malignant Œdema.

1862 DAVAINE.—Bull. de l'Acad. de Méd.

1877 PASTEUR.—Vibrion. Septique. Bull. de l'Acad. de Méd.

1881 KOCH.—Mitth. aus dem Ges. Amt, I., S. 54.

GAFFKY.—Mitth. as. d. K. Ges. Amt.

PASTEUR.—Vibrion. Septique. Bull. de l'Acad. de Méd.

1882 BRIEGER U. EHRLICH.—Berl. Klin. Wochenschr., Nr. 44.

LEBEDEFF.—Versuche über Desinfection bei Malignen Oedembacillen. Arch. de Phys. Norm. et Path.

1884 LUSTIG.—Zur Kenntniss Bakteriämischer Erkrankungen bei Pferden (Malignes Oedem). Jahresber. d. K. Thierarzneischule zu Hannover.

KITT.—Unters. über Malignes Oedem und Rauschbrand bei Hausthieren. Jahresber. der K. Thierarzneischule in München.

1885 HESSE, W. U. R.—Ueber Züchtung der Bacillen des Malignen Oedems. Deut. Med. Woch.

(XVIII.) Measles.

1882 KEATING.—Phil. Med. Times.
1883 CORNIL ET BABÈS.—Archiv de Phys.

(XIX.) Ophthalmic Diseases.

1872 RÖTH.—Retinitis Septica. Virchow's Archiv, Bd. 55.
1879 BALOGH.—Sphærobakterien in der entzündeten Hornhaut. Med. Centralbl., XIV.
1882 KAHLER.—Ueber Septische Retinitis. Prager Zeitschr. f. Prakt. Heilk., Bd. I.
SATTLER.—Unters. über das Trachom. Ber. üb. d. Ophthalmologen Congress zu Heidelberg.
MICHEL.—Graefe's Archiv f. Augenheilkunde.
1883 SCHLEICH.—Verh. des Ophthalmologen Congr. zu Heidelberg.
BOCK.—Ueber die Miliare Tuberkulose der Uvea. Virchow's Arch., Bd. 91.
KUSCHBERT U. NEISSER.—Zur Pathologie und Aetiologie der Xerosis Conjunctivæ. Bresl. Aerztl. Zeitschr., Nr. 4.
SATTLER.—Ueber d. Natur der Jequirity-Ophthalmie. Zehender's Klin. Monatsblatt; and Wien. Med. Woch., Nr. 17.
SATTLER ET DE WECKER.—L'Ophthalmie Jéquiritique.
CORNIL ET BERLIOZ.—Sur l'Empoisonnement par le Jéquirity. Compt. Rend. de l'Acad. d. Sc.
1884 v. REUSS.—Pilzconcretionen i. d. Thränenröhrchen. Wien. Med. Presse.
GOLDZIEHER.—Streptothrix Fœrsteri im unteren Thränenröhrchen. Centralblatt f. Prakt. Augenheilk.

1884 KRONER.—Zur Aetiologie der Ophthalmoblennorrhœ. Verh. d. Naturforscher-Vers. Magdeburg.
KUSCHBERT.—Deutsch. Med. Woch., Nr. 21.
VOSSIUS.—Berl. Klin. Wochenschr., Nr. 17.
KLEIN.—Centralbl. f. d. Med. Wiss., Nr. 8.
VENNEMANN ET BRUYLANTS.—Le Jéquirity et son Principe Pathogène.
NEISSER.—Fortschr. d. Med., Bd. 2, S. 73.
SALOMONSEN.—Fortschr. d. Med., Bd. 2, S. 78.
1885 WIDMARK.—Hygeia. Stockholm.
DEUTSCHMANN.—v. Graefe's Archiv, Bd. XXXI.
1886 GIFFORD.—Archiv f. Augenheilkunde.
v. ZEHENDER.—Bowman Lecture. Lancet

(XX.) Osteomyelitis.

1873 COLLMANN.—Bakterien im Organismus eines an einer Verletzung am Oberschenkel verstorbenen Mädchens. Göttingen.
1875 EBERTH.—Virchow's Arch., Bd. 65.
1876 FRIEDMANN.—Fall von Primärer Infectiöser Osteomyelitis. Berl. Klin. Woch.
SCHÜLLER.—Zur Kenntniss der Mikrokokken bei Acuter Infectiöser Osteomyelitis. Mikrokokkenherde im Gelenkknorpel. Centralbl. f. Chirurgie, Nr. 12.
1882 FEHLEISEN.—Phys. Med. Ges. Würzburg.
1883 BECKER.—Vorl. Mitth. über den die Acute Infectiöse Osteomyelitis erzeugenden Mikroorganismus. Deut. Med. Woch., and Berl. Klin. Woch.
1884 PEYROUD.—Compt. Rend.
KRAUSE.—Ueber einen bei der Acuten Infectiösen Osteomyelitis vorkommenden Mikrokokkus. Fortschr. d. Med., Bd. 2.
ROSENBACH.—Vorl. Mitth. über die die Acute Osteomyelitis beim Menschen erzeugenden Mikroorganismen. Centralbl. f. Chirurgie.

1884 RODET.—Compt. Rendus, T. 99.
1885 GARRÉ.—Fortschr. d. Med.

(XXI.) Pleuropneumonia.

1879 SUSSDORFF.—Ueber d. Lungenseuche des Rindes. Deutsche Zeitschr. f. Thiermed. u. Vergl. Pathol.
1880 BRUYLANTS ET VERRIERS. — Bull. de l'Acad. Belg.
1882 PASTEUR.—Note sur la Péripneumonie Contagieuse des Bêtes à Cornes. Recueil de Méd. Vét.
1883 CORNIL ET BABÈS.—Arch. de Physiol. Norm. et Path., T. 2.
1884 POELS U. NOLEN.—Centralbl. f. d. Med. Wiss., Nr. 9.
MAYRWIESER.—Ueber Infectiösen Bronchialcroup bei Rindern. Woch. f. Thierheilk. u. Viehzucht, 19.
1886 POELS U. NOLEN.—Das Contagium der Lungenseuche. Fortsch. d. Med.
POELS.—Septische Pleuro-pneumonia der Kälber. Fortsch. d. Med.

(XXII.) Pneumonia.

1878 KÜHN.—Die Contagiöse Pneumonie. Deutsch. Arch. f. Klin. Med.
1880 BRUYLANT ET VERRIERS.—Bull. de l'Acad. Belge.
1881 KÜHN.—Die Uebertragbarkeit Endemischer Pneumonieformen auf Kaninchen. Berl. Klin. Wochenschr., Nr. 38.
1882 FRIEDLÄNDER.—Virchow's Arch., Bd. 87.
1883 SALVIOLI U. ZÄSLEIN.—Ueber den Micrococcus und die Pathogenese der Croupösen Pneumonie. Centralbl. f. d. Med. Wissensch.
MATRAY.—Wien. Med. Presse, Nr. 23.

1883 ZIEHL.—Ueber das Vorkommen der Pneumoniekokken im Pneumonischen Sputum. Centralbl. f. d. Med. Wissensch.

FRIEDLÄNDER.—Fortschr. d. Med., Bd. I.

TALAMON.—Progr. Médic.

GILES.—Brit. Med. J., Vol. II.

FRIEDLÄNDER U. FROBENIUS.—Berl. Klin. Woch.

GRIFFINI AND CAMBRIA.—Centralbl. f. d. Med. Wiss.

1884 FRIEDLÄNDER.—Fortschr. d. Med., Bd. II.

ZIEHL.—Ueber das Vorkommen der Pneumoniekokken im Pneumonischen Sputum. Centralbl. f. d. Med. Wissensch.

KLEIN.—Centralbl. f. d. Med. Wissensch.

NAUWERCK.—Ueber Morbus Brightii bei Croupöser Pneumonie. Beitr. zur Pathol. Anat. von Ziegler.

JÜRGENSEN.—Berl. Klin. Wochenschr., Bd. 22.

MENDELSSOHN.—Zeitschr. f. Klin. Med., Bd. 7.

PLATONOW.—Ueber die Diagnostische Bedeutung d. Pneumoniekokken. Inaug.-Diss. Würzburg.

KORÁNYI U. BABÈS.—Pester Med. Chirurg. Presse.

A. FRÄNKEL.—Verhandl. d. Congr. f. Innere Med., Fortschr. d. Med. Nov.

GERMAIN-SÉE.—Compt. Rend. Acad. de Sc. Paris.

AFANASSIEW.—Compt. Rend. Soc. de Biol. Paris. T. 5.

SALVIOLI ET ZÀSLEIN.—Arch. pour les Sc. Méd., T. 8.

MAGUIRE.—Brit. Med. Journ., Vol. II.

1885 DRESCHFELD.—Ueber Wanderpneumonie u. ihre Beziehung zur Epidemischen Pneumonie. Fortschr. d. Med., Bd. 3, 389.

SCHOU.—Untersuchungen über Vaguspneumonie Fortschr. der Medicin, Bd. 3, Nr. 15.

RIBBERT.—Zur Färbung d. Pneumoniekokken. Deut. Med. Wochenschr., Nr. 9.

1885 GERMAIN-SÉE.—Des Maladies Spécifiques du Poumon.
DE BLASI.—Rivista Internaz. di. Med. e. Chir.
PAWLOWSKY.—Berl. Klin. Woch.
PLATANOW.—Mitth. a. d. Würzburg. Med. Klinik.
STERNBERG.—Amer. Journ. Med. Sciences.
PETERLEIN.—Bericht ü. d. Vet.-Wesen. i. K. Sachsen.
FRIEDLÄNDER.—Beste Färbung zur Darstellung der Kapseln der Pneumoniekokken. Fortschritt, Bd. 3, 92.
1886 STERNBERG.—Micrococcus Pasteuri. Journ. Royal Microscop. Soc.
NEUMANN.—Berl. Klin. Woch.
PIPPING.—Fortsch. d. Med. Nos. 10 and 14.
FRÄNKEL.—Zeitschr. f. Klin. Med. Bd. X. and XI.
FRÄNKEL.—Deut. Med. Woch.
FOA U. BORDONI-UFFREDUZZI.—Deut. Med. Woch.
WECHSELBAUM.—Wien. Med. Woch.
MANFREDI.—Fortsch. d. Med.
JACCOUD.—La France Médicale.
LANCEREAUX ET BESANÇON.—Archiv. Gén. de Méd.
THOST.—Deut. Med. Woch.

(XXIII.) Puerperal Fever.

1865 MAYRHOFER.—Vibrionen als Krankheitsursache des Puerperalfiebers. Monatsschr. f. Geburtsk. u. Frauenkrankheiten, Bd. 25.
1872 WALDEYER.—Ueber Vorkommen von Bakterien bei der Diphtheritischen Form des Puerperalfiebers. Arch. f. Gynäkologie, III.
1873 HEIBERG.—Die Puerperalen und Pyämischen Processe.
ORTH.—Virchow's Arch., Bd. 58.

1873 RECKLINGHAUSEN U. LANKOWSKI.—Ueber Erysipelas. Virchow's Arch., Bd. 60.
1879 LAFFTER.—Gehirnerweichungsherde durch Mikrokokkenembolieen bei Puerperaler Pyämie. Bresl. Aerztl. Ztg.
1880 DOLÉRIS.—La Fièvre Puerpérale et les Organismes Infect.
PASTEUR.—Bull. de l'Acad. de Méd., T. 9.
1881 KAREWSKI.—Exper. Unters. über die Einwirkung Puerperaler Secrete auf den Thierischen Organismus. Zeitschr. f. Geburtsh. u. Gynäkologie, Bd. 7.
1884 AUFRECHT.—Die Exper. Erzeugung der Endometritis Diphtherica Puerperalis. Naturforsch. Versamml.

(XXIV.) Pyæmia, Septicæmia, and Suppuration.

1866 RINDFLEISCH.—Lehrb. der Pathol. Gewebelehre. 1. Aufl., S. 204.
1867 WALDEYER.—Zur Pathol. Anatomie der Wundinfectionskrankheiten. Virchow's Arch., Bd. 40.
LISTER.—Lancet.
1870 AINSTIE.—Lancet.
1871 TIEGEL.—Ueber d. Fiebererregenden Eigenschaften des Microsporon Septicum. Bern. Diss.
WALDEYER.—Mikrokokkencolonien in den Parenchymatischen Organen. Vortrag i. d. Med. Ges. zu Breslau. 4 Aug.
1872 BURDON-SANDERSON.—Trans. Path. Soc.
1873 BIRCH-HIRSCHFELD.—Untersuchungen über Pyämie.
HEIBERG.—Die Puerperalen u. Pyämischen Processe.

1874 TIEGEL.—Virchow's Archiv, Bd. 60.
1875 BARTHOLD.—Pyæmisch-Metast. Dissert. Berlin.
BURDON-SANDERSON.—Brit. Med. Journ.
1877 BALFOUR.—Edinb. Med. Journ.
LISTER.—Med. Times and Gazette.
1878 DOWDESWELL.—Quart. Journ. Micr. Sc. London, Vol. 18.
KOCH.—Wundinfectionskrankheiten. Leipzig.
BASTIAN.—Brit. Med. Journ.
1880 PERRET.—De la Septicémie. Paris.
BECK.—Rep. Loc. Govt. Board.
DRYSDALE.—Pyrexia.
1881 KOCH.—Mittheil. d. Kais. Ges. Amts, Bd. I.
OERTEL.—Zur Aetiologie der Infectionskrankheiten.
OGSTON.—Brit. Med. Journ., Vol. I.
BÉCHAMP.—Compt. Rend.
BÉCHAMP.—Trans. Internat. Med. Cong. London.
HORSLEY.—Rep. Med. Officer Loc. Govt. Board.
LISTER.—Quart. Journ. Microscop. Science.
1882 OGSTON.—Journ. of Anat. and Phys., Vol. 17.
BRAIDWOOD AND VACHER.—Brit. Med. Journ.
STERNBERG.—Amer. Journ. Med. Sc.,—John Hopkins' Univ. Stud. Biol. Lab.
1883 DOWDESWELL.—Proc. Roy. Soc. London, Vol. 34.
BABÈS.—Compt. Rend.
DRESCHFELD.—Brit Med. Journ.
SUTTON.—Trans. Path. Soc.
1884 ROSENBACH.—Mikroorganismen bei den Wundinfectionskrankheiten des Menschen. Wiesbaden.
ARLOING.—Recherches sur les Septicémies.
WATSON-CHEYNE.—Trans. Path. Soc., XXXV.
STEVEN.—Glasgow Med. Journal.
1885 PASSET.—Ueber Mikroorganismen der Eitrigen Zellgewebsentzündung des Menschen. Fort schritte d. Med., Nr. 2.

1885 GARRÉ.—Zur Aetiologie acut Eitriger Entzündungen. Fortschritte d. Med., 165.
PASSET.—Fortschritte d. Medicin, Bd. 3.
KLEMPERER.—Zeitschr. f. Klin. Med.
HOFFA.—Fortsch. d. Med.

(XXV.) Relapsing Fever.

1873 OBERMEIER.—Vorkommen Feinster, Eigene Bewegung zeigender Fäden im Blute von Recurrenskranken. Med. Centralbl., 11. Berl. Med. Ges. Berl. Klin. Wochenschr.
ENGEL.—Ueber die Obermeier'schen Recurrensspirillen. Berl. Klin. Woch.
1875 LAPTSCHINSKY.—Centralbl. f. d. Med. Wissensch., Bd. 13.
1876 WEIGERT.—Deut. Med. Woch.
HEYDENREICH.—St. Petersb. Med. Woch.
MANASSEM.—S. Petersb. Med. Woch., No. 18.
1877 HEYDENREICH.—Der Parasit des Rückfalltyphus.
1879 CARTER.—Lancet.
ALBRECHT.—St. Petersb. Med. Woch.
COHN.—Deut. Med. Woch.
KOCH.—Deut. Med. Woch.
1880 GUTTMANN.—Virchow's Arch.
MÜHLHAUSER.—Virchow's Arch., Bd. 97.
JAKSCH.—Wien. Med. Wochenschr. Juli.
1885 GÜNTHER.—Fortschr. d. Med.

(XXVI.) Rhinoscleroma.

1885 CORNIL.—B. de la Soc. Anatom. 15 Fev.
CORNIL ET ALVAREZ.—Sur les Micro-organismes du Rhinosclérome. Acad. de Méd. et Archiv. de Phys. Norm. et Path.
1886 PALTAUF U. EISELSBERG.—Fortsch. d. Med.
DAVIES.—Brit. Med. Journ.

(XXVII.) Scarlatina.

1872 COZE ET FELTZ.—Les Maladies Infectieuses.
MCKENDRICK.—Brit. Med. Journal.
1876 KLEIN.—Report of the Medical Officer of the Privy Council.
1882 HAHN.—Berl. Klin. Woch., No. 38.
1883 ROTH.—Münchener Aerztl. Intelligenzbl.
POHL-PINCUS.—Centralbl. f. d. Med. Wiss., No. 36.
CROOKE.—Lancet.
1884 HEUBNER U. BAHRDT.—Zur Kenntniss der Gelenkeiterungen bei Scharlach. Berl. Klin. Woch., Nr. 44.
1885 CROOKE.—Fortsch. d. Med.
1886 LAURE.—Lyon Médical.
KLEIN.—Nature, XXXIV.

(XXVIII.) Swine-Erysipelas.

1881 SALMON.—Report Depart. Agricul. Washington.
DETMERS.—Science.
1882 PASTEUR.—Sur le Rouget ou Mal Rouge des Porcs. Compt. Rend., T. 95.
1883 CORNIL ET BABÈS.—Arch. de Physiol.
PASTEUR ET THUILLIER.—Bull. de l'Acad. de Méd. de Paris. Compt. Rend., T. 97.
EGGELING.—Ueber den Rothlauf der Schweine. Fortschr. d. Med.
1884 SALMON.—Rep. Dept. Agricult. Washington.
1885 LÖFFLER.—Experimentelle Untersuchungen über Schweinerothlauf. Arbeiten aus dem Kaiserl. Gesundheits Amt, Bd. I.
SCHÜTZ.—Ueber den Rothlauf der Schweine und die Impfung desselben. Ibid.
LYDTIN U. SCHOTTELIUS.—Der Rothlauf der Schweine. Wiesbaden.

(XXIX.) Swine-Typhoid.

1879 KLEIN.—Rep. of the Med. Offic. of the Privy Council [1877-1878].

1884 KLEIN.—Virchow's Archiv.

(XXX.) Symptomatic Anthrax.

1878 BOLLINGER U. FESER.—Wochenschr. f. Thierheilkunde.

1880 ARLOING, CORNEVING ET THOMAS.—Compt. Rend.

1881 ARLOING, CORNEVING ET THOMAS.—Bull. de l'Acad. de Méd. and Revue de Méd.

1883 ARLOING, CORNEVING ET THOMAS.—Du Charbon Bactérien, Charbon Symptomatique, etc.

1884 KITT.—Unters. über Malignes Oedem und Rauschbrand bei Hausthieren. Jahresber. der K. Thierarzneisch. in München.

BABÈS.—Journ. de l'Anatomie.

NEELSEN U. EHLERS.—Ueber den Rauschbrand. Ber. d. Naturforsch. Ges. zu Rostock.

EHLERS.—Unters. üb. d. Rauschbrandpilz. Inaug. Diss. Rostock.

ARLOING, CORNEVING ET THOMAS.—Revue de Méd.

1886 ARLOING, CORNEVING ET THOMAS.—Chabert's Disease. Transl. by Dawson Williams in Microparasites and Disease. (New Syd. Soc.)

HESS.—Bericht über die entschädigten Rauschbrand u. Milzbrandfälle im Canton Bern.

STREBEL.—Schweiz. Archiv f. Thierheilk.

(XXXI.) Syphilis.

1872 LOSTORFER.—Arch. f. Dermat. u. Syph.
1879 KLEBS.—Arch. f. Exp. Pathol., Bd. 10.
1881 AUFRECHT.—Centralbl. f. d. Med. Wissensch., Bd. 19.
1882 BIRCH-HIRSCHFELD.—Bakterien in Syphilitischen Neubildungen. Centralbl. f. d. Med. Wissensch., Nrs. 33, 34.
PESCHEL.—Centralbl. f. Augenheilk.
MARTINEAU ET HAMONIC.—De la Bactéridie Syphilitique. Compt. Rend., p. 443.
MORISON.—Maryland Med. Journ.
1883 LETNIK.—Wien. Med. Wochenschr.
MORISON.—Maryland Med. Journ. Baltimore.
MORISON.—Wiener Med. Wochenschr.
HEYDEN.—Préservation de la Syphilis, etc. Traduit par Roberts.
1884 PETRONE.—Gaz. Medica Ital.
TORNEY ET MARCUS.—Compt. Rend., p. 472.
LUSTGARTEN.—Wien. Med. Woch., Nr. 47.
KÖNIGER.—Deut. Med. Woch., S. 816.
1885 LUSTGARTEN.—Die Syphilisbacillen.
DOUTRELEPONT U. SCHÜTZ.—Deut. Med. Woch., Nr. 19.
ALVAREZ ET TAVEL.—Bull. de l'Acad. de Méd. et Archiv. de Phys. Norm. et Path.
DE GIACOMI.—Neue Färbungsmethode der Syphilisbacillen. Correspondenzbl. f. Schweizer Aerzte, Bd. 15.
GOTTSTEIN.—Fortschr. d. Med., Bd. 3, S. 543.
KLEMPERER.—Deut. Med. Woch.
1886 EVE and LINGARD.—Brit. Med. Journ.
BIENSTOCK.—Fortsch. d. Med.
KASSOWITZ U. HOCHSINGER.—Wien. Med. Blätter.
DISSE U. TAGUCHI.—Deut. Med. Woch.

(XXXII.) Tetanus.

1884 CARLE E RATTONE. — Studio Sperimentale sull' Etiologia del Tetano. Giorn. della R. Acad. di Medicina di Torino.
VOGEL.—Drei Fälle von Infectiösem Tetanus. Deut. Med. Woch., Nr. 31.
NICOLAIER.—Deut. Med. Woch., Nr. 52.
1886 ROSENBACH.—Archiv. f. Klin. Chir.

(XXXIII.) Tuberculosis.

1868 VILLEMIN.—Etude sur la Tuberculose.
KLEBS.—Virchow's Arch., Bd. 44.
1873 KLEBS.—Arch. f. Exp. Pathol. u. Pharmakol., Bd I.
1877 COHNHEIM.—Uebertragbarkeit der Tuberculose. Berlin.
1879 BAUMGARTEN.—Berl. Klin. Woch., Bd. 17.
1881 TOUSSAINT.—Compt. Rend., T. 93.
BAUMGARTEN.—Centralbl. f. d. Med. Wiss. Berlin. Bd. 19.
1882 BAUMGARTEN.— Centralbl. f. d. Med. Wiss. Berlin. Bd. 20.
BAUMGARTEN.—Deut. Med. Woch., Bd. 8.
WAHL.—Zur Tuberculosefrage. Deut. Med. Woch., Nr. 46.
VAN ERMENGEM.—Le Microbe de la Tuberculose. Ann. de la Soc. Belge de Microscopie.
HILLER. Deut. Med. Woch., Bd. 8.
BALOGH.—Wien. Med. Woch.
KOCH.—Die Aetiologie der Tuberculose. Berl. Klin. Woch.
FORMAD.—The Bacillus Tuberculosis. The Philad. Medical Times.
GREEN.—Lancet.

1882 CREIGHTON.—Trans. Path. Soc.
RINDFLEISCH.—Phys. Med. Ges. zu Würzburg, Nr. 8.
EWART.—Lancet.
EHRLICH.—Deut. Med. Woch.
FRÄNTZEL U. PALMERS.—Berl. Klin. Woch.
AUFRECHT.—Centralbl. für d. Med. Wiss.
1883 SCHUCHARDT U. KRAUSE.—Fortschr. d. Med.
KLEBS.—Arch. f. Exp. Pathol. u. Pharmakol., Bd. 17.
EHRLICH.—Deut. Med. Woch., Bd. 9.
FRÄNTZEL U. PALMERS.—Berl. Klin. Woch.
DE GIACOMI.—Fortschr. d. Med., Bd. I, S. 145.
MEISELS.—Wien. Med. Woch.
RAYMOND.—Arch. Gén. de Méd., T. 11.
DEMME.—Jahresber. d. Jennerschen Kinderspitals. Bern.
LYDTIN.—Badische Thierärztl. Mittheil.
RIBBERT.—Ueber d. Verbreitungsweise der Tuberkelbacillen bei Hühnern. Deut. Med. Woch., Nr. 28.
WECHSELBAUM.—Wien. Med. Jahrb.
JOHNE.—Ber. üb. d. Veterinärwesen im Königr. Sachsen.
BOLLINGER.—Münch. Aerztl. Intelligenzbl., Nr. 16.
JOHNE.—Die Geschichte der Tuberculose.
PÜTZ.—Ueber die Beziehungen der Tuberculose des Menschen zu der der Thiere.
CELLI and GUARNERI.—Arch. pour les Sciences Médic.
WEIGERT.—Deut. Med. Woch. Nr. 24ff.
NAUWERCK.—Deut. Med. Woch.
SMITH.—Bristol. Med. Chir. Journ.
WILLIAMS.—Lancet.
VERAGUTH.—Arch. f. Exp. Path. u. Pharmakol., Bd. 16.
SCHOTTELIUS.—Virchow's Archiv, Bd. 91.

1883 BROUILLY.—Note sur la Présence des Bacilles dans les Lésions Chirurgicales Tuberculeuses. Rev. de Chir., T. 3.
SCHLEGTENDAL.—Fortschr. d. Med., Bd. I.
KÜSSNER.—Beitr. z. Impftuberculose. Deut. Med. Woch., Nr. 36.
AUFRECHT.—Centralbl. f. d. Med. Wiss., Bd. 21.
BOLLINGER.—Centralbl. f. d. Med. Wiss., Bd. 21, S. 600.
CHEYNE.—Brit. Med. Journ., Vol. I.
CHEYNE.—Practitioner. London. Vol. XXX.
BOCK.—Virchow's Archiv, Bd. 91.
DETTWEILER.—Berl. Klin. Woch., Bd. 21.
DAMSCH.—Deut. Med. Woch., Nr. 17.
BABÈS.—Der erste Nachweis des Tuberkelbacillus im Harn. Centralbl. f. d. Med. Wissensch.
BABÈS.—Compt. Rendus.
ROSENSTEIN.—Centralbl. f. d. Med. Wiss.
LEVINSKY.—Deut. Med. Woch., Bd. 9.
LICHTHEIM.—Fortschr. d. Med., Bd. I.
WEST.—Lancet, Vol. I.
WEST.—Trans. Path. Soc.
ZIEHL. — Bedeutung der Tuberkelbacillen für Diagnose und Prognose. Deut. Med. Woch., Nr. 5.
DIEULAFOY ET KRISHABER.—Arch. de Physiol. Norm. et Path., T. I.
MALASSEZ ET VIGNAL. — Ibid., T. II. Compt. Rend., T. 97.
KOCH.—Kritische Besprechung der gegen die Bedeutung der Tuberkelbacillen gerichteten Publicationen. Deut. Med. Woch., Nr. 10.
VIGNAL.—Compt. Rend. Soc. de Biol., T. 5.
GIBBES.—Lancet.
KUNDRAT.—Wien. Med. Presse.
LANDOUZY ET MARTIN.—Rev. de Méd., T. 3.
CHIARI.—Wien. Med. Presse.

1883 COCHET.—Compt. Rend. Soc. de Biol., Paris, T. 5.
PFEIFFER.—Berlin. Klin. Woch., Bd. 21.
LEUBE. — Sitzungsber. der Phys.-Med. Soc. zu Erlangen.
SPINA.—Studien über Tuberculose. Wien.
CRAMER.—Sitzungsber. d. Phys. Med. Soc. zu Erlangen.
MARCHAND.—Deut. Med. Woch., Nr. 15.
HERON.—Lancet.
GREEN.—Brit. Med. Journ.
BAUMGARTEN.—Centralbl. f. d. Med. Wissensch. Berlin. Bd. 21.
BAUMGARTEN.—Zeitschr. f. Klin. Med., Bd. 6.
SOMARI E BRUGNATELLI. — Redii R. Instit. Lombardo.
1884 MALASSEZ ET VIGNAL.—Sur le Microorg. de la Tuberculose Zoogloeïque. Compt. Rend., T. 99, p. 200.
DOUTRELEPONT.—Die Aetiologie des Lupus Vulgaris. Vierteljahrschr. f. Dermatologie u. Syphilis.
CORNIL ET LELOIR. — Recherches, etc., sur la Nature du Lupus. Arch. de Physiol. Norm. et Pathol.
BABÈS ET CORNIL.—Note sur les Bacilles de la Tuberculose. Journ. de l'Anat. et de la Physiol. Norm. et Pathol.
BOULEY.—La Nature Vivante de la Contagion. Contagiosité de la Tuberculose. Paris.
DÉJÉRINE.—Rev. de. Méd. Paris. T. 4.
ARLOING.—Compt. Rend.
BIEDERT.—Virchow's Archiv, Bd. 98.
LEYDEN. — Klinisches über Tuberkelbacillen. Zeitschr. f. Klin. Med., VIII.
MÜLLER.—Ueber den Befund von Tuberkelbacillen bei Fungösen Knochen u. Gelenkaffectionen. Centralbl. f. Chir., 3.
ALBRECHT.—Arch. f. Kinderheilk., Bd. 5.

1884 SCHILL U. FISCHER.— Mitth. a. d. Kaiserl. Ges. Amt, Bd. II.

LUSTIG.—Ueber Tuberkelbacillen im Blut bei an Allg. Acuter Miliartub. Erkrankten. Wien. Med. Woch., Nr. 48.

VOLTOLINI. — Ueber Tuberkelbacillen im Ohr. Deut. Med. Woch., Nr. 31.

STRASSMANN.—Virchow's Archiv, Bd. 96.

KOCH.—Mittheilungen aus dem Kais. Ges. Amt, Bd. II.

BAUMGARTEN.—Centralbl. f. d. Med. Wissensch. Berlin. Bd. 22.

GAFFKY.—Verhalten der Tuberkelbacillen im Sputum. Mitth. a. d. Kaiserl. Gesundheitsamt, Bd. II.

NEGRI.—Colorations des Spores dans les Bacilles de la Tuberculose. Journ. de Microsc., T. 8.

SUTTON.—Trans. Path. Soc. London, Vol. XXXV.

ANDREW.—Lancet.

WILLIAMS.—Journ. Royal Microscop. Soc.

1885 DOUTRELEPONT. — Fall von Meningitis Tuberculosa nach Lupus, Tuberkelbacillen im Blut. Deut. Med. Woch., Nr. 7.

KARG.—Centralbl. f. Chir.

SPINA.—Casopis Lekaru Ceskych, Nr. 4.

OBRZUT.—Prof. Spina's Neue Färbungsmethode der Fäulnissmikroorganismen und ihre Beziehung zu den Tuberkelbacillen. Deut. Med. Woch., Nr. 12.

CREIGHTON.—Brit. Med. Journ.

CREIGHTON.—Lancet.

FÜTTERER.—Ueber das Vorkommen u. die Vertheilung der Tuberkelbacillen in den Organen. Virch. Arch., Bd. 100, Heft 2.

JOHNE.—Ein Zweifelh. Fall von Congenitaler Tuberculose. Fortschr. d. Med., Bd. 3, 198.

BIEDERT.—Berl. Klin. Woch.

RIBBERT.—Deut. Med. Woch.

1885 HARRIS.—St. Barthol. Hosp. Reports.
STICKER.—Centralbl. f. Klin. Med.
WESENER.—Fütterungstuberculose.
TSCHERNING.—Inoculationstuberculose beim Menschen. Fortschr. d. Med., Bd. 3, 65.
1886 MIDDELDORPF.—Fortsch. d. Med.
MÜLLER.—Centralbl. für Chir.
GOLDENBLUM.—Vrach., Nos. I. and XI.
BLACK.—Lancet.
BAUMGARTEN.—Zeitschrift. f. Klin. Med.
KIRSTEIN.—Deut. Med. Woch.
MAX BENDER.—Ueber die Beziehungen des Lupus Vulg. zu Tuberc. Deut. Med. Woch.
HARRIES AND CAMPBELL.—Lupus. London.

(XXXIV.) Typhoid Fever.

1874 BIRCH-HIRSCHFELD.—Unters. zur Pathologie des Typhus Abdominalis. Zeitschr. f. Epidemiologie, I.
KLEIN.—Med. Centralbl., XII.
1876 LETZERICH.—Virchow's Archiv, Bd. 68.
1877 FELTZ.—Compt. Rend., T. 85.
1878 LETZERICH.—Archiv f. Exper. Pathol., Bd. 9.
1880 EPPINGER.—Beitr. zur Pathol. Anatomie aus d. Patholog. Institut Prag.
FISCHEL.—Beitr. zur Pathol. Anat. aus d. Pathol.-Anat. Inst. Prag.
1880 TIZZONI.—Studi. di Pat. Sperim. sulla Gen. d. Tifo. Milano.
KLEBS.—Arch. f. Exper. Pathol. u. Pharmakol.
EBERTH.—Arch. f. Pathol. Anat., Bd. 81.
1881 LETZERICH.—Archiv f. Exper. Pathol., Bd. 10.
BRAUTLECHT.—Pathogene Bakterien im Trinkwasser bei Epidemieen. Virchow's Arch., Bd. 84.

1881 MEYER.—Unters. über den Bacillus des Abdominaltyphus. Inaug. Diss.
RAPPIN.—Contrib. à l'Etude des Bact. de la Bouche, à l'Etat Normal et dans la Fièvre Typhoïde.
1882 COATS.—Eberth's Typhoïd Bacillus. Brit. Med. Journ.
WERNICH.—Zeitschr. f. Klin. Med., Bd. 6.
MARAGHANO.—Centralbl. f. d. Med. Wiss., Bd. 15.
ALMQUIST.—Typhoïdfeberus-Bakterie.
CROOKE.—Brit. Med. Journ.
1883 BOENS.—Acad. Roy. de Méd. de Belgique. Bull., 3 Sér., T. 17.
EBERTH.—Der Typhus-Bacillus und die Intestinelle Infection. Volkmann, Klin. Vorträge.
LETZERICH.—Experimentelle Untersuchungen über die Aetiologie des Typhus Abdominalis. Leipzig.
1884 GAFFKY.—Zur Aetiologie des Addominaltyphus. Mitth. a. d. Ges. Amt, Bd. II.
TAYON.—Le Microbe de la Fièvre Typhoïde de l'Homme. Compt. Rend., T. 99, p. 331.
1885 PFEIFFER.—Ueber den Nachweis der Typhusbacillen im Darminhalt u. Stuhlgang. Deut. Med. Woch., Nr. 29.
1886 MICHAEL. — Typhusbacillen im Trinkwasser. Fortsch. d. Med.
MEISELS.—Wien. Med. Woch.
SEITZ.—Bakteriolog. Studien z. Typhusätiologie.
LUCATELLO.—Bollet. d. R. Accad. Med. di Genova.
FRAENKEL U. SIMMONDS.—Die Aetiologische Bedeutung des Typhus-Bacillus.
NEUHAUSS.—Berl. Klin. Woch.
SIROTININ.—Zeitschr. f. Hygiene.
BEUMER AND PEIPER.—Zeitschr. f. Hygiene.

(XXXV.) Variola and Vaccinia.

1868 CHAUVEAU.—Nature du Virus-Vaccin. Compt. Rend.
1871 WEIGERT.—Ueber Bakterien in der Pockenhaut.
1872 COHN.—Virchow's Archiv, Bd. 55.
ZÜLZER.—Berl. Klin. Wochenschr.
1873 LUGINBUHL.—Der Micrococcus der Variola. Verhdl. d. Phys. Med. Ges. in Würzburg.
1874 WEIGERT.—Anat. Beitr. z. Lehre v. d. Pocken.
PISSIN.—Berl. Klin. Wochenschr.
STROPP.—Vaccination u. Mikrokokken.
KLEBS.—Arch. f. Exp. Path. u. Pharmakol., Bd. 10.
1880 ISCHAMER.—Ueber das Wesen des Contag. der Variola Vaccine u. Varicella. Aerztl. Verein. Steiermark.
1881 TAPPE.—Aetiologie u. Histologie der Schafpocken.
1882 POHL-PINCUS.—Vaccination.
PFEIFFER.—Ueber die Rückimpfung auf Kühe und Kälber. Jahrb. f. Kinderheilk.
MARCHAND.—Les Virus-Vaccins. Revue Mycologique.
STRAUSS.—Vaccinal Micrococci. Soc. de Biol. de Paris.
1883 WOLF.—Zur Impffrage. Berl. Klin. Wochenschr., Nr. 4.
PLAUT.—Das Organis. Contagium der Schafpocken.
CORNIL ET BABÈS.—Soc. Médicale des Hôpitaux.
QUIST.—St. Petersburg Medic. Woch., Nr. 46.
1884 HAMERINK.—Ueber die sog. Vaccination u. Variola.

(XXXVI.) Yellow Fever.

1883 BABÈS.—Sur les Microbes trouvés dans le Foie et dans le Rein d'Individus morts de la Fièvre Jaune. Compt. Rend., 17 Sept.

1884 DOMINGOS FREIRE ET REBOURGEON.—Compt. Rend., T. 99, p. 804.
DOMINGOS FREIRE.—Recherches sur la Cause de la Fièvre Jaune.
1885 BOULEY.—L'Inoculation Préventive de la Fièvre Jaune. Compt. Rend., T., 100, p. 1276.
CARMONA Y VALLE.—Leçons sur l'Etiol. et la Prophylax. de la Fièvre Jaune.
1886 CERECEDO.—El Siglo Medico.

(K.) *BACTERIA IN THE AIR, IN SOIL, AND IN WATER.*

1858 POUCHET.—Compt. Rend., T. 47.
1860 PASTEUR.—Compt. Rend., T. 50.
1861 PASTEUR.—Compt. Rend., T. 52.
1862 PASTEUR.—Ann. de Chim. et de Phys., T. 64.
1863 PASTEUR.—Compt. Rend., T. 56.
LEMAIRE.—Compt. Rend., T. 57.
1870 TYNDALL.—Med. Tim. and Gaz.
MADDOX.—Month. Microscop. Journal.
1874 CUNNINGHAM.—Micr. Exam. of the Air. Calcutta.
TISSANDIER.—Comp. Rend., T. 78.
1877 PASTEUR.—Compt. Rend., T. 85.
MIQUEL.—Annuaire de l'Observat. de Montsouris.
TYNDALL.—Brit. Med. Journ.
1878 MIQUEL.—Compt. Rend., T. 86.
MIQUEL.—De la Présence dans l'Air du Ferment de l'Urée. Bull. de la Soc. Chim.
1879 MIFLET.—Unters. über die in der Luft Suspendirten Bakterien. Cohn's Beitr. z. Biol. d. Pflanzen, Bd. III.
WERNICH.—Cohn's Beiträge z. Biol. d. Pflanzen, Bd. III., S. 105.
KLEBS U. TOMMASI-CRUDELI.—Unters. der Luft auf die Mikroorganismen der Malaria. Archiv f. Exper. Path., Bd. 11.

1879 MIQUEL.—Ann. d'Hygiène.
1881 KOCH.—Milzbrandbacillen im Boden. Mitth. a. d. Ges. Amt, Bd. I.
SOYKA.—Sitz.-Ber der K. Bayr. Akad. d. Wiss. Math. Physik. Classe.
SOYKA.—Vorträge im Aerztl. Verein in München.
BÜCHNER.—Die Bedingungen des Uebergangs von Pilzen in die Luft. Vorträge im Aerztl. Verein zu München.
TYNDALL.—Essays on the Floating Matter of the Air.
1882 WINNACKER.—Ueber die in Rinnsteinen beobacht. Nied. Organismen. Göttinger Inaug.-Diss. Frankfurt a. M.
v. FODOR.—Hygienische Unters. über Luft, Boden u. Wasser.
NÄGELI.—Unters. über Niedere Pilze.
SOYKA.—D. Vierteljsch. f. Oeff. Ges., Bd. 14.
MIQUEL.—Annuaire de l'Observat. de Montsouris.
1883 WOLLNY.—Ueber die Thätigkeit Niederer Organismen im Boden. Viert. f. Oeff. Ges., S. 705.
LETZERICH.—Exp. Unters. üb. die Aetiologie des Typhus mit bes. Berücksichtigung der Trink- u. Gebrauchswässer.
ZANDER.—Centralbl. f. Allg. Ges.
GUNNING.—Beitr. z. Hygienischen Untersuchung des Wassers, Arch. f. Hyg., 3.
MIQUEL.—Les Organismes Vivants de l'Atmosphère.
OLIVIER.—Les Germes de l'Air. Thèse. Rev. Scientif.
ANGUS SMITH.—Sanitary Record.
SMART.—Germs, Dust, and Disease.
TORELLI.—La Malaria in Italia.
1884 HESSE.—Ueber Quantitative Best. der in der Luft enthaltenen Mikroorganismen. Mitth. a. d. Ges. Amt, Bd. II.

1884 HESSE.—Ueber Abscheidung d. Mikroorganismen aus d. Luft. Deutsche Med. Wochenschr., 2.
HESSE.—Weitere Mitth. über Luftfiltration. Deut. Med. Woch., Nr. 51.
SCHRAKAMP.—Archiv f. Hygiene, Bd. II.
MIQUEL ET FREUDENREICH.—La Semaine Médicale.
SEHLEN.—Fortschr. d. Med., Bd. II, S. 585.
CHAMBERLAND.—Sur un Filtre Donnant de l'Eau Physiologiquement Pure. Compt. Rend., T. 99, p. 247.
MOREAU ET PLANTYMAUSION.—La Semaine Médicale.
ANGUS SMITH.—On the Examination of Waters. Rep. to the Loc. Gov. Board.
1885 CRAMER.—Die Wasserversorgung von Zürich.
CROOKSHANK.—Notes from a Bact. Labor., Lancet.
BECKER.—Reichsmedicinalkalender.
HESSE.—Ueber Wasserfiltration. Deut. Med. Woch.
SOYKA.—Prager Med. Woch.
FRANKLAND.—Removal of Micro-organism from Water. Proc. Roy. Soc.
1886 SOYKA.—Fortschr. d. Med.
LAURENT.—Journal de Pharmacie et de Chemie.
WOLFFHÜGEL AND RIEDEL.—Arbeit. a. d. K. Ges. Amt.
HERÆUS.—Zeitschr. f. Hygiene.
C. FRÄNKEL.—Zeitschr. f. Hygiene.
MEADE BOLTON.—Zeitschr. f. Hygiene.
HESSE.—Zeitschr. f. Hygiene.
BEUMER.—Deut. Med. Woch.
FRANKLAND.—Proc. Roy. Soc.
BISCHOF.—Journ. Soc. Chem. Industry.
PFEIFFER.—Zeitschr. f. Hygiene.

APPENDIX G.

TABLE SHOWING THE MAGNIFYING POWER OF ZEISS' OBJECTIVES.

Ocular:	1	2	3	4	5	
a_1	7	11	15	22		a_1
a_2	12	17	24	34		a_2
a_3	20	27	38	52		a_3
a*		4—12	7—17	10—24		a*
aa	22	30	41	56	75	aa
A, AA	38	52	71	97	130	A, AA
B, BB	70	95	130	175	235	B, BB
C, CC	120	145	195	270	360	C, CC
D, DD	175	230	320	435	580	D, DD
E	270	355	490	670	890	E
F	405	540	745	1010	1350	F
G	260	340	470	640	855	G
H	320	430	590	805	1075	H
J	430	570	785	1070	1430	J
K	570	760	1045	1425	1900	K
L	770	1030	1415	1930	2570	L
$\frac{1}{8}$	260	340	470	640	855	$\frac{1}{8}$
$\frac{1}{12}$	380	505	695	950	1265	$\frac{1}{12}$
$\frac{1}{18}$	605	810	1110	1515	2020	$\frac{1}{18}$
	1	2	3	4	5	

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PLATE I.

FIGS. 1 to 39.

Bacteria, Schizomycetes, or Fission fungi.

(Facing Title-page.)

For description, see p. xxiv.

PLATE II.

FIG. 1.—Bacterium indicum.

Micrococcus indicus.
Bacillus indicus.

Tube inoculated from a nutrient agar-agar plate-cultivation. By plate-cultivation, or by successive cultivation on potatoes, a *pure* cultivation can be obtained. The growth has then the colour of red sealing-wax, and a peculiar crinkled appearance. After some days the growth loses its bright colour, and becomes purplish like an old cultivation of *Bacterium prodigiosum.*

FIG. 2.—Bacillus cyanogenus.

Bacterium syncyanum.
Bacillus of blue milk.

Tube inoculated from a potato-cultivation. The bacillus forms a whitish layer, and colours the nutrient agar-agar a smoky brown.

FIG. 3.—Bacterium prodigiosum.

Monas prodigiosa.
Micrococcus prodigiosus.
Bacillus prodigiosus.
Blood rain.

Tube inoculated from a potato cultivation. The bacterium grows very rapidly, forming a blood-red growth, which gradually acquires a purplish colour.

CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1 Micrococcus urbeus
Fig 2 Bacillus cyaneus
Fig 3 Micrococcus prodigiosus

PLATE III.

FIG. 1.—Spirillum choleræ asiaticæ.

Comma-bacillus of Koch.

Tube inoculated from a plate-cultivation. The growth in this case was very striking. The funnel-shaped area of liquefaction, enclosing an air-bubble, and the white thread along the needle track, are in marked contrast to the appearances, under similar conditions, of the comma-bacillus in Cholera nostras. (Fig. 96.)

FIG. 2.—Bacterium choleræ gallinarum.

Micrococcus choleræ gallinarum.
Microbe du choléra des poules.

Tube inoculated from the blood of a hen which had died of so-called chicken-cholera. After several days the growth forms a very delicate, finely beaded thread.

FIG. 3.—Streptococcus cereus albus.

Staphylococcus cereus albus.

Tube inoculated from the pus of a subcutaneous abscess in a rabbit. The growth assumed a nodular appearance along the needle track.

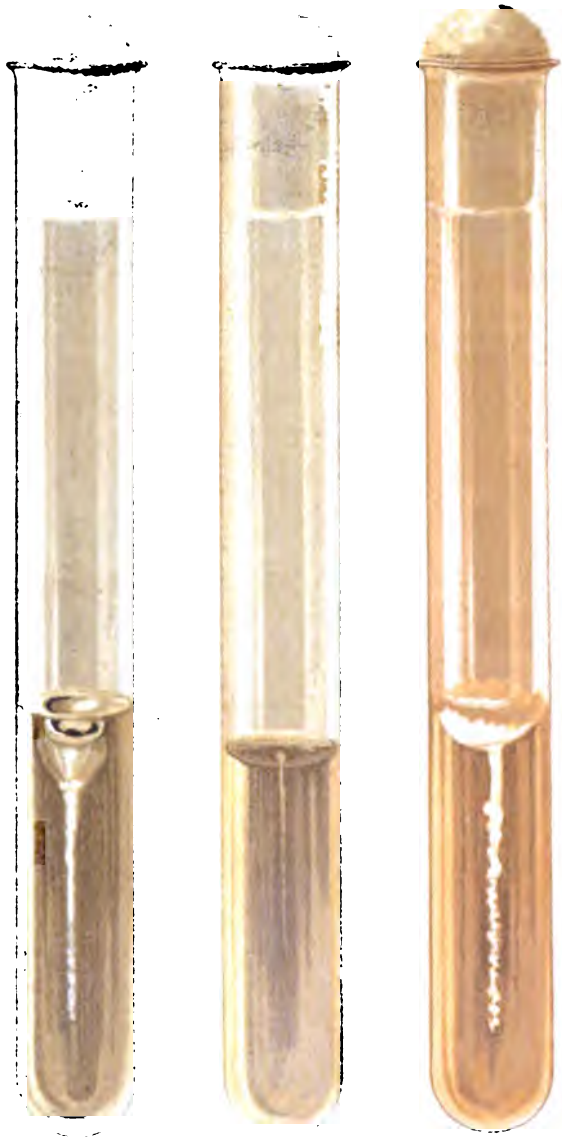

Fig 1 Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE

Fig 1 Spirillum choleræ asiaticæ.
Fig 2 Micrococcus choleræ gallinarum.
Fig 3. Staphylococcus cereus albus

PLATE IV.

FIG. 1.—Micrococcus tetragonus.

Tube inoculated from a plate-cultivation of bacteria in sputum. The cultivation consisted of a milk-white growth heaped up on the surface of the gelatine and growing freely along the upper part of the needle track.

FIG. 2.—Bacterium pneumoniæ crouposæ.

Micrococcus pneumoniæ crouposæ.
Friedländer's pneumo-coccus.

Tube inoculated from pneumonic exudation. The growth, in nutrient gelatine, in the form of a round-headed nail is not by itself distinctive.

FIG. 3.—Saccharomyces niger.

Black torula.

Tube inoculated from an old contaminated nutrient gelatine cultivation. The growth, isolated and re-inoculated, formed a black crust on the surface of the gelatine. In some of the tubes little separated centres of growth occurred in the upper part of the track of the needle.

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE.

Fig 1. Micrococcus tetragonus

Fig 2. Bacterium pneumoniæ crouposæ

PLATE V.

FIG. 1.—Bacillus pyocyaneus.

Bacterium æruginosum.
Bacillus fluorescens.

Tube inoculated from pus. The gelatine was liquefied and appeared green by transmitted and orange by reflected light.

FIG. 2.—Sarcina lutea.

Tube inoculated from a colony which occurred on potato exposed to the air. The gelatine was partially liquefied, and a canary-yellow growth had subsided to the bottom of the liquefied layer.

FIG. 3.—Bacillus anthracis.

Tube inoculated from the blood of a mouse which had died of anthrax. The typical growth which occurs in a few days is shown in Fig. 107. In this figure the appearance after three weeks is represented. The gelatine was completely liquefied, and a flocculent mass had subsided to the bottom of the tube.

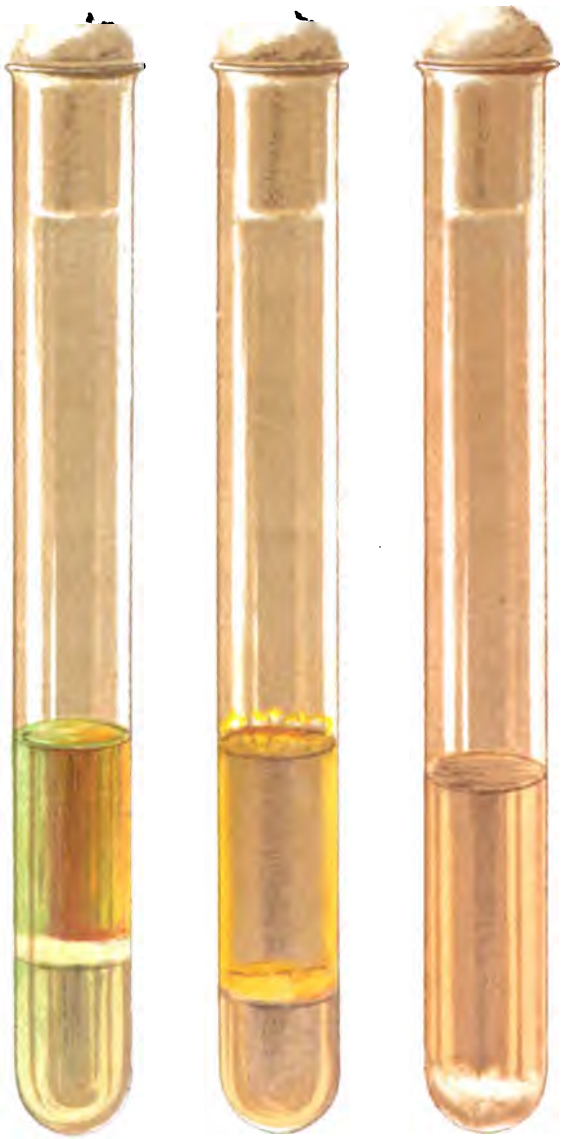

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE

Fig 1 Bacterium aeruginosum.
Fig 2. Sarcina lutea.
Fig 3. Bacillus anthracis

PLATE VI.

Spirillum Finkleri.

Comma-bacillus of Finkler and Prior.

This figure represents the appearance of a plate-cultivation of the comma-bacillus from Cholera nostras, when examined over a slab of blackened plate-glass. The colonies differ very markedly from the colonies of Koch's comma-bacilli (see Fig. 85). The drawing was made from a typical result of thinning out or attenuating* the colonies by the process of plate-cultivation. At this stage they were completely isolated one from the other; but later they became confluent and produced complete liquefaction of the gelatine.

* The term "attenuation" is applied also to a virus, in the sense of weakening or modifying its effect. To avoid confusion the term *mitigation* might be employed exclusively to express this, and *attenuation* used only in the sense indicated above.

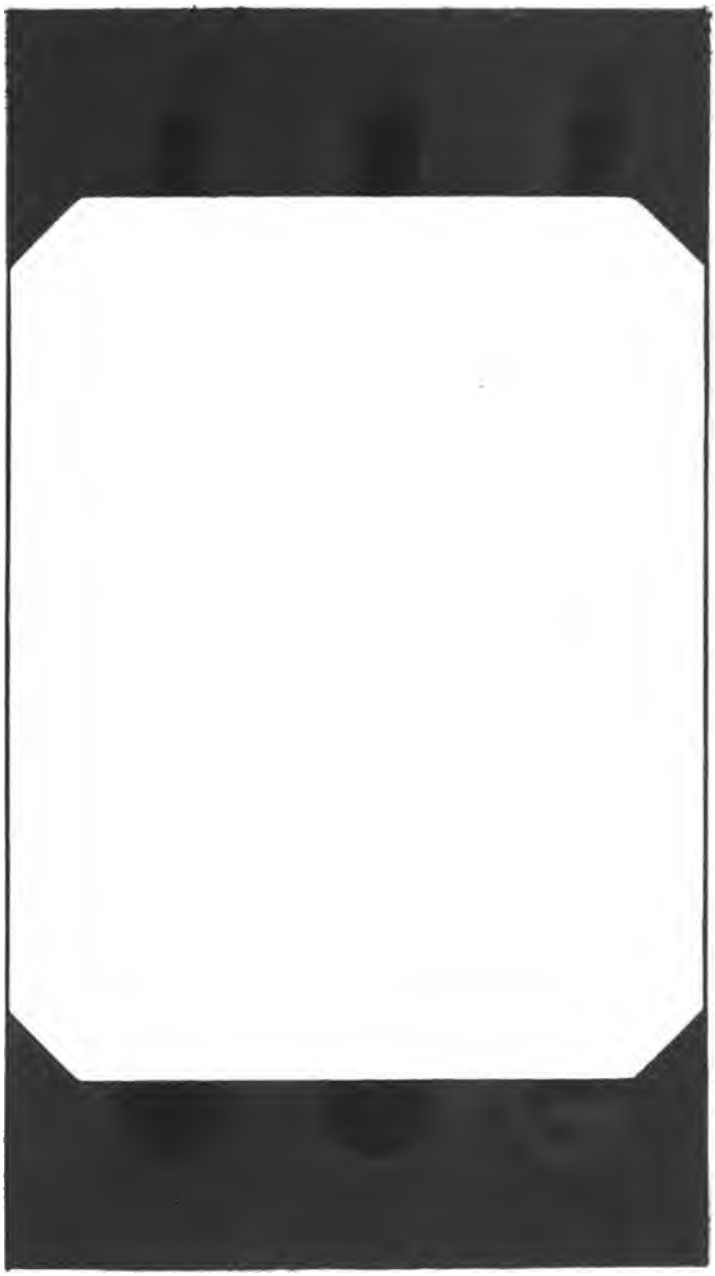

PLATE - CULTIVATION.

First attenuation of the Spirillum Finkleria, after twenty-four hours.

PLATE VII.

Spirillum Finkleri.

Comma-bacillus of Finkler and Prior.

This figure represents the result obtained by a still further thinning out of the organisms than in the preceding case. The attenuation had been so far carried out that several of the colonies remained completely isolated for days.

PLATE 7.

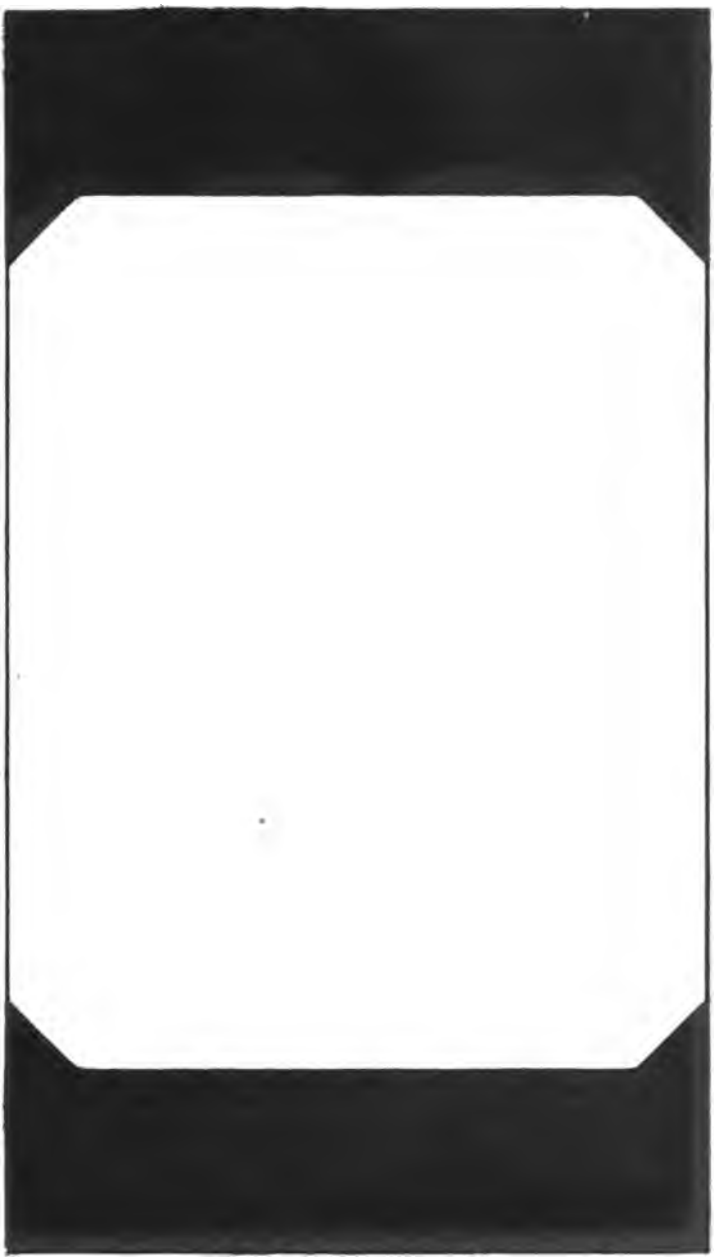

PLATE CULTIVATION.

Second attenuation of the Spirillum Finkleri. after thirty-six hours.

PLATE VIII.

FIG. 1.—Sarcina lutea.

Tube of nutrient agar-agar inoculated from a plate-cultivation. The canary-yellow colour forms a strong contrast to the colour of the growth in the adjacent tube.

FIG. 2.—Streptococcus pyogenes aureus.

Staphylococcus pyogenes aureus.
Micrococcus pyogenes aureus.

Tube inoculated from an abscess in a rabbit.

FIG. 3.—Bacillus pyocyaneus.

Bacillus fluorescens.
Bacterium aeruginosum.

Tube inoculated from a colony on a plate-cultivation. The growth formed a whitish, transparent layer composed of slender bacilli. The pigment diffused itself throughout the nutrient jelly. The growth appears green by transmitted light owing to the colour of the medium behind it. The bacillus is now regarded as identical with the bacillus of green-blue pus.

PLATE 6.

CULTIVATIONS ON NUTRIENT AGAR-AGAR.

Fig 1. Sarcina lutea.
Fig 2. Micrococcus pyogenes aureus.
Fig 3. Bacillus fluorescens.

PLATE IX.

FIG. 1.—Bacterium prodigiosum.

Monas prodigiosa.
Micrococcus prodigiosus.
Bacillus prodigiosus.
Blood rain.

Growth on potato after three days.

FIG. 2.—Penicillium glaucum.

A potato which had been freely exposed to the air was covered in three weeks by a growth of *Penicillium glaucum*. The surface of the growth is studded with dew-like drops of moisture.

Fig 1. Micrococcus prodigiosus.
Second attenuation after three days

Fig 2. Penicillium glaucum.
after three weeks growth.

POTATO CULTIVATIONS.

London published by H.K. Lewis, 136 Gower Street.

PLATE X.

FIG. 1.—Sarcina lutea.

Growth on sterilised potato five days after inoculation from a tube-cultivation. Potatoes, especially old ones, have sometimes a tendency to become discoloured, and the brown appearance in this figure has nothing to do with the growth of the organisms.

FIG. 2.—Saccharomyces rosaceus.

Pink torula.

Growth on sterilised potato which had been inoculated from a colony contaminating a plate-cultivation. This yeast develops a coral-pink colour, but does not grow so luxuriantly as the chromogenic bacteria.

Fig 1. Sarcina lutea
Growth five days after inoculation.

Fig 2. Saccharomyces rosaceus.
Growth two days after inoculation

POTATO CULTIVATIONS.

PLATE XI.

Bacillus tuberculosis.

FIG. 1.

Pure cultivation of the tubercle-bacillus on blood-serum solidified obliquely.

FIG. 2.

Pure cultivation on solid blood-serum in a glass capsule.

FIG. 3.

The same as Fig. 2, examined under a low power of the microscope. × 80.

FIG. 4.

Impression-preparation showing the peculiar serpentine growth of the colonies on blood-serum. × 700.

(After Koch, *Mittheil. a. d. Kaiserl. Gesundh. Amt.*)

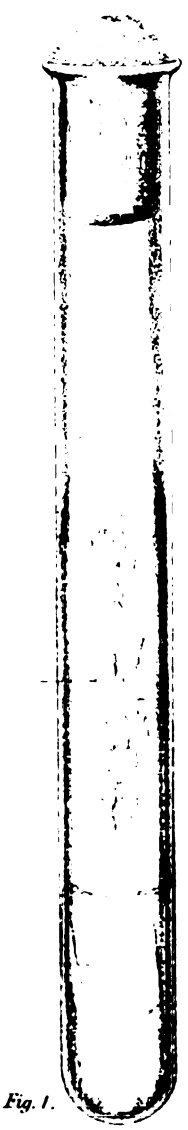

Fig. 1.

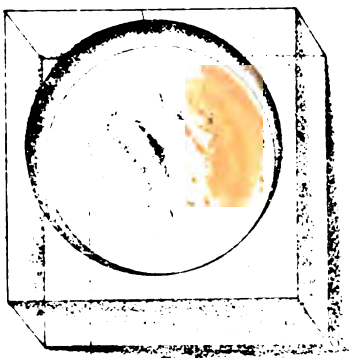

Fig. 2.

Fig. 3.

Fig. 4.

BACILLUS TUBERCULOSIS.

Fig. 1. Pure-cultivation on solid blood serum in a test-tube.
Fig. 2. Pure-cultivation on solid blood serum in a glass-capsule.
Fig. 3. The same preparation as Fig. 2 (x 80).
Fig. 4. Cover-glass impression preparation of colonies x 700 (Ehrlichs method)

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PLATE XII.

FIG. 1.—Micrococcus tetragonus.

From a section of a kidney of a mouse which had died in eight days, after inoculation subcutaneously with a pure cultivation. Encapsuled tetrads, isolated and in masses, were found in the kidneys, lungs, and other organs. Stained with Gram's method (gentian violet) without a contrast stain. × 1,500.

FIG. 2.—Streptococcus pyogenes aureus.

Micrococcus pyogenes aureus.
Staphylococcus pyogenes aureus.

From a section of the liver of a rabbit. A small vessel is shown plugged with cocci. From small abscesses in the liver, cultivations were obtained of the characteristic yellow coccus of pus. Stained with Gram's method (gentian violet) without a contrast stain. × 1,500.

MICROCOCCUS TETRAGONUS.
Fig. 1. From a section of Kidney of a mouse.
Gram's method. Zeiss' 1/8 o.i. Oc. 4.

MICROCOCCUS PYOGENES AUREUS.
Fig. 2. From a section of Liver of a rabbit
Gram's method. Zeiss' 1/8 o. i. Oc. 4.

PLATE XIII.

FIG. 1.—**Sarcina lutea.**

In this tube and the two adjacent ones, the inoculations were made by thrusting the needle into the nutrient agar-agar. In all three cases the growth on the surface, freely exposed to air, developed a characteristic pigment, while the growth in the track of the needle was scanty and colourless.

FIG. 2.—**Bacterium indicum.**

Micrococcus indicus.
Bacillus indicus.

FIG. 3.—**Saccharomyces rosaceus.**

Pink torula.

Fig 1. Fig 2. Fig 3

CULTIVATIONS IN NUTRIENT AGAR-AGAR

Fig 1. Sarcina lutea.
Fig 2. Micrococcus indicus.
Fig 3. Saccharomyces rosaceus.

PLATE XIV.

FIG. 1.—Bacillus anthracis.

Bactéridie du charbon.
Bacillus of splenic fever, woolsorter's disease, or malignant pustule.

Tube of nutrient agar-agar inoculated with the blood of a sheep which had died of anthrax. White flocculent patches developed, which were entirely composed of threads and spores of the bacilli.

FIG. 2.—Bacillus subtilis.

Tube inoculated with bacilli, isolated by plate-cultivation, from dust. The bacilli appeared to be identical with the hay bacillus, but in this case formed a peculiar crinkled layer along the track of the needle.

FIG. 3.—Streptococcus cereus albus.

Staphylococcus cereus albus.

Tube inoculated from the discharge of a subcutaneous abscess in a rabbit.

CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1 Bacillus anthracis.
Fig 2. Bacillus subtilis.
Fig 3. Staphylococcus cereus albus.

PLATE XV.

FIG. 1.—Bacillus anthracis.

Bactéridie du charbon.
Bacillus of splenic fever, woolsorter's disease or malignant pustule.

The bacillus of anthrax grows very rapidly on sterilised potato, especially when placed in the incubator at the temperature of the blood. The growth forms a creamy-yellow layer, with copious spore formation.

FIG. 2.—Bacterium indicum.

Micrococcus indicus.
Bacillus indicus.

Sterilised potato inoculated with a pure growth obtained by successive cultivations. Unless the growth is quite free from the presence of other bacteria, the brilliant red colour is not obtained.

PLATE 15

Fig 1. Bacillus anthracis.
Growth at 37° C three days after inoculation.

Fig 2. Micrococcus indicus.
Growth three days after inoculation.

POTATO CULTIVATIONS.

H. Beckerhaak Photo.

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PLATE XVI.

Bacillus anthracis.

Bactéridie du charbon.
Bacillus of splenic fever, woolsorter's disease, or malignant pustule.

FIG. 1.

From a section of the mucous membrane of the stomach of a mouse. The glandular capillaries are mapped out by the bacilli. Stained by the method of Gram (gentian violet), and eosin. × 500.

FIG. 2.

From a section of a kidney of a mouse. Under a low power the preparation has exactly the appearance of an injected specimen. Under higher amplification, the bacilli are seen to have threaded their way along the capillaries between the tubules, and to have collected in masses in the glomeruli. Stained with Gram's method (gentian violet), and eosin. × 500.

PLATE 16.

Fig. 1. From a section of mucous membrane of the stomach of a mouse. Gram's method and Eosin. Zeiss' ik. o. i. Oc. 2.

BACILLUS ANTHRACIS.

Fig. 2. From a section of Kidney of a mouse. Gram's method and Eosin Zeiss' ik. o. i. Oc. 2.

PLATE XVII.

FIG. 1.—Bacillus anthracis.

Bactéridie du charbon.
Bacillus of splenic fever, woolsorter's disease, or malignant pustule.

From a section of the liver of a mouse which had died after inoculation with a pure cultivation of the bacillus. The bacilli are seen to have threaded their way between the liver-cells. The preparation is triple-stained by combining the methods of Weigert and Orth. × 500.

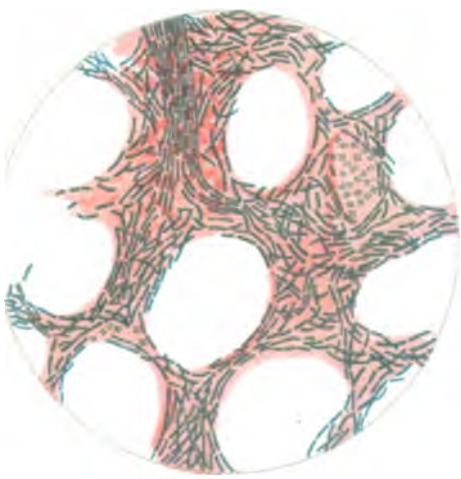
FIG. 2.—Bacillus anthracis and Micrococcus tetragonus.

From a section of a lung of a mouse which had been inoculated with anthrax three days after inoculation with *Micrococcus tetragonus*. A double or mixed infection resulted. Anthrax bacilli occurred in vast numbers, completely filling the small vessels and capillaries, and in addition there were great numbers of the characteristic tetrads. Stained with Gram's method (gentian violet), and eosin. × 500.

PLATE 17.

BACILLUS ANTHRACIS.

Fig. 1. From a section of Liver of a mouse. Weigert's and Orth's methods. (gentian-violet and piero-lithium-carmine.) Zeiss' 1/12 o.i. Oc. 2.



BACILLUS ANTHRACIS AND MICROCOCCUS TETRAGONUS.

Fig. 2. From a section of Lung of a mouse. Gram's method and Eosin. Zeiss' 1/12 o.i. Oc. 2

PLATE XVIII.

Bacillus tuberculosis.

FIG. 1.

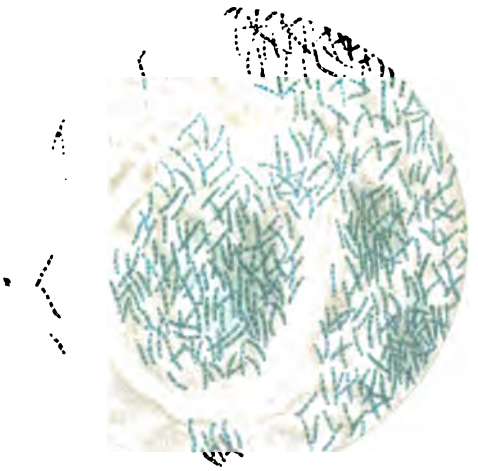
From a section of a lymphatic gland of a fœtal calf. The preparation was stained by the Ehrlich-Koch method (methyl violet and bismarck brown), and eosin. The giant cell takes the eosin stain, the nuclei are stained brown, and the bacilli blue. In the interior of the giant cell are numerous coloured grains, the significance of which is not known, and a number of tubercle bacilli. × 1,500.

For the material from which this preparation was made the author is indebted to Professor Johne, by whom an account of this case was published, "Ein Zweifelloser Fall von Congenitaler Tuberkulose," *Fortsch. d. Med.*, 1885, No. 7, p. 198.

FIG. 2.

From a section of a lung of a rabbit after inoculation with tubercular sputum. Caseous areas are seen, and masses of bacilli showing distinct beading. Stained by the Ehrlich-Koch method (methyl violet) without a contrast stain. × 1,500.

Fig. 1. From a section of a lymphatic gland from a case of tuberculosis in a foetal calf. Ehrlich-Koch method. (methyl-violet and bismarck-brown) and Eosin. Zeiss' 12. o.i. Oc. 4.



BACILLUS TUBERCULOSIS.

Fig. 2. From a section of Lung from a case of artificial tuberculosis in a rabbit. Ehrlich-Koch method. (methyl-violet) Zeiss' 18 o.i. Oc. 4.

PLATE XIX.

Bacillus tuberculosis.

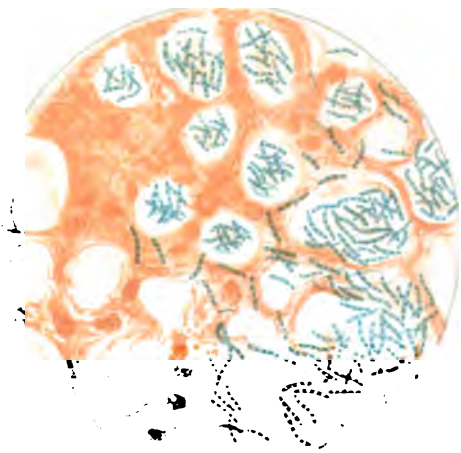
FIG. 1.

From a section of the liver of a tubercular hen. With a moderate power the areas of caseation and the topographical distribution of the bacilli can be studied. Stained with the Ehrlich-Koch method (methyl violet and bismarck brown). × 400.

FIG. 2.

From the same preparation with high amplification, showing that the parts stained blue consist entirely of bacilli. × 1,500.

Fig. 1. From a section of Liver of a hen.
Ehrlich-Koch method (methyl-violet and bismarck-brown) Zeiss' DD. Oc. 4.



BACILLUS TUBERCULOSIS.
Fig. 2. The same preparation. Zeiss' B. Oc. 4

PLATE XX.

FIG. 1.—**Bacillus tuberculosis.**

From a cover-glass preparation of tubercular pus. Stained with Ehrlich's method (fuchsine and methylene blue). × 1,500.

FIG. 2.—**Bacillus leprae.**

From a section of a kidney from a case of leprosy. Stained with Ehrlich's method (fuchsine and methylene blue). In the centre of the field is a glomerulus with a collection of the leprosy bacilli. × 400.

PLATE 20.

BACILLUS TUBERCULOSIS.

Fig 1. From a cover-glass preparation of pus from a tubercular cavity of the human lung. Ehrlich's method (fuchsine and methylene blue). Zeiss' Ib. o.i. Oc 4.

BACILLUS LEPRÆ.

Fig. 2. From a section of kidney from a case of leprosy. Ehrlich's method. (fuchsine and methylene blue). Zeiss' DD. Oc. 4.

PLATE XXI.

Bacillus cyanogenus.

Bacterium syncyanum.
Bacillus of blue milk.

FIGS. 1 and 2.

Potato inoculated from a cultivation in nutrient gelatine. In three days a peculiar bluish-green growth develops on the surface of the potato, and in nine days it has a heaped-up margin of a bluish-green colour, while the central portion has turned almost black.

PLATE 21.

Fig 1. After three days growth.

Fig 2 After nine days growth.

POTATO CULTIVATION OF BACILLUS CYANOGENUS.

PLATE XXII.

Bacillus of septicæmia of mice.

FIG. 1.

From a section of a kidney of a mouse which had died after inoculation with a pure cultivation of the bacillus. With moderate amplification, the white blood-corpuscles have a granular appearance, and irregular granular masses scattered between the kidney tubules are seen. Stained with Gram's method and eosin. × 200.

FIG. 2.

Part of the same preparation with high amplification. The granular appearances are found to be due to the presence of great numbers of extremely minute bacilli. × 1,500.

PLATE 22.

Fig. 1. From a section of Kidney of a mouse. Gram's method and Eosin. Zeiss' DD. Oc. 2.

BACILLUS OF SEPTICÆMIA OF MICE.

Fig. 2. The same preparation. Zeiss' ⅛. o i. Oc. 4.

PLATE XXIII.

Bacillus lepræ.

FIG 1.

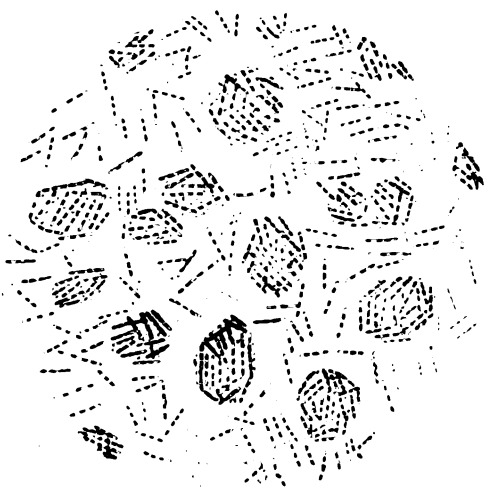
From a section of the skin of a leper. The section is, almost in its entirety, stained purple, and, with moderate amplification, has a finely granular appearance. Stained with Ehrlich's method (fuchsine and methylene blue). × 200.

FIG. 2.

Part of the same preparation with high amplification, showing that the appearances described above are due entirely to an invasion of the tissue by the bacilli of leprosy. × 1,500.

PLATE 23.

Fig 1. From a section of skin from a case of leprosy. Ehrlich's method (fuchsine and methylene blue.) Zeiss AA Oc. 2.



BACILLUS LEPRÆ.

Fig 2. The same preparation. Zeiss' 1/12 oil. Oc 4

PLATE XXIV.

FIG. 1.—**Bacterium lineola.**

Tube inoculated from putrid blood-serum which swarmed with *Bacterium lineola.* The same bacteria were found in the cultivation.

FIG. 2.—**Micrococcus rosaceus.**

Tube inoculated from an old cultivation on nutrient agar-agar which had become contaminated. The cultivation, macroscopically resembling pink yeast, consisted of a pure cultivation of micrococci.

FIG. 3.—**Streptococcus pyogenes citreus.**

Tube inoculated with cocci isolated from a subcutaneous abscess in a mouse.

CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1 Bacterium lineola.
Fig 2 Micrococcus roseus.
Fig 3 Staphylococcus pyogenes citreus

PLATE XXV.

Bacillus figurans.

Bacillus mycoides.
Wurzel Bacillus.

FIG. 1.

From an impression-preparation of a growth on the surface of nutrient gelatine. × 50.

FIG. 2.

Part of the same preparation with high amplification, showing that the coils and filaments of the growth are due to a peculiar and regular arrangement of the individual bacilli. × 1,500.

Fig. 1. Cover-glass impression-preparation from a plate-cultivation. (fuchsine). Zeiss' A.A. Oc. 2.

BACILLUS FIGURANS.

Fig. 2. The same preparation. Zeiss' ⅛ o.i. Oc 4

Edgar Crookshank fec. et pinx. London, Published by H. K. Lewis, 136, Gower Street Vincent Brooks Day & Son Lu

PLATE XXVI.

Bacillus of swine-erysipelas.

Bacillus of German swine-fever.

FIG. 1.

Pure cultivation in nutrient gelatine. The growth is stated to be identical with that of the bacillus of septicæmia in mice.

FIG. 2.

Colonies on a plate-cultivation.

FIG. 3.

Cover-glass preparation of blood from an inoculated pigeon.

(After Schütz, *Arb. a. d. Kais. Gesundh. Amt*, Bd. I.)

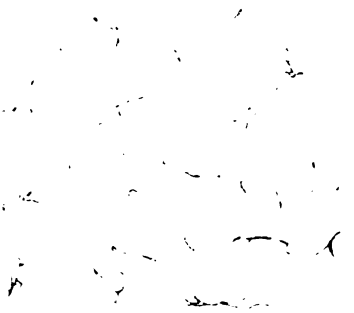


Fig. 2.

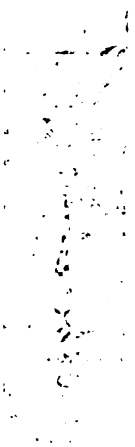


Fig. 1.

Fig. 3.

BACILLUS OF SWINE-FEVER (SCHÜTZ).

Fig. 1. Pure-cultivation in nutrient gelatine.
Fig. 2. Colonies on a plate-cultivation.
Fig. 3. Cover-glass preparation of blood from an inoculated pigeon

PLATE XXVII.

Actinomyces.

FIG. 1.

From a section of a maxillary tumour in a cow. Stained by Plaut's method (magenta and picric acid). × 90.

FIG. 2.

Part of the same preparation, with higher amplification. The fungoid masses are very deeply stained by this method. The component club-shaped elements and their radiate arrangement are clearly shown. × 500.

PLATE 27.

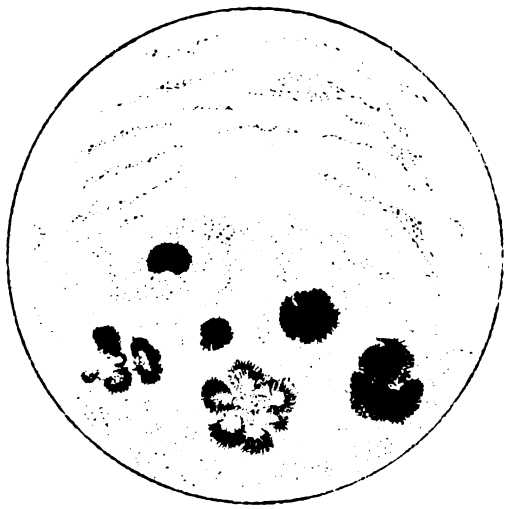


Fig. 1. From a section of a maxillary tumour in a cow. Plaut's method (Magenta and picric acid). Zeiss' A. Oc. 4.

ACTINOMYCES.

Fig. 2. The same preparation. Zeiss' A. o. i. Oc. 2.

PLATE XXVIII.

Actinomyces.

FIG. 1.

From a section of a maxillary tumour in a cow. Stained by Weigert's method (orseille and gentian violet). × 900.

FIG. 2.

From a section of the lung of a cow. The rosettes are much smaller, possibly owing to their being more confined by their surroundings than when growing in the soft pulpy tissue of the maxillary tumour. They are here shown with high amplification, but under a power of about 50 diam. (Zeiss A.A. Oc. 2) the section of a lung resembles miliary tuberculosis, and in the centre of a neoplasm the rosette appears about the size of a pin's head. Stained with Weigert's method (orseille and gentian violet). × 500.

PLATE 28.

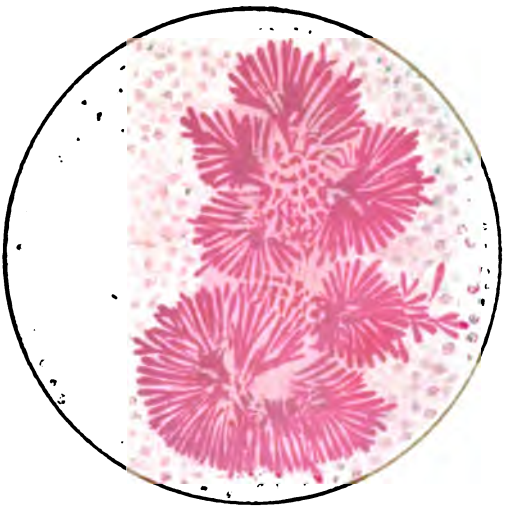
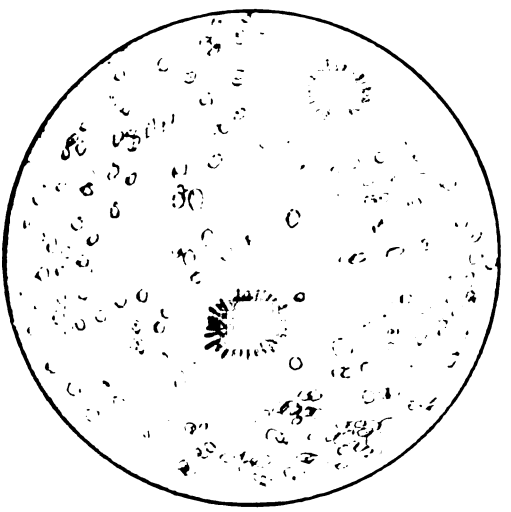


Fig. 1. From a section of a maxillary tumour in a cow.
Weigert's method. (Orseille and gentian-violet.) Zeiss' H. o i. Oc. 4.



ACTINOMYCES.

Fig. 2. From a section of the lung of a cow.
Weigert's method. (Orseille and gentian-violet.) Zeiss' H. o i. Oc. 2.

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PLATE XXIX.

FIGS. 1 to 11.

Yeast-fungi or saccharomycetes and mould fungi or hyphomycetes.

(Facing page 339.)

For description see p. 339.

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